Is bacterial translocation involved in the pathogenesis of idiopathic Non Cirrhotic Intrahepatic Portal Hypertension?

A dissertation submitted in partial fulfillment of the requirements for

DM (Branch IV, Gastroenterology) examination of the

Tamil Nadu Dr. M.G.R. Medical University, Chennai,

to be held in August 2013.

Certificate

This is to certify that this dissertation entitled **"Is bacterial translocation involved in the pathogenesis of idiopathic Non Cirrhotic Intrahepatic Portal Hypertension?"** is a bonafide work done by Dr. Alagammai PL in partial fulfillment of the rules and regulations for DM (Branch IV – Gastroenterology) examination of The Tamil Nadu Dr MGR Medical University, to be held in August 2013.

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Place: Vellore

Date:

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Is bacterial translocation involved in the pathogenesis of idiopathic Non Cirrhotic Intrahepatic Portal Hypertension? by Alagammai Palaniappan 16102751 D.M. Medical Gastroenterology

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CONTENTS

INTRODUCTION

Non cirrhotic intrahepatic portal hypertension (NCIPH) is traditionally named as non cirrhotic portal fibrosis (NCPF) in India. It is more prevalent in developing countries and it is a commonly encountered clinical entity. As per data from our centre, cryptogenic cirrhosis is the commonest cause for portal hypertension, 39 to 48% of patients labeled as cryptogenic cirrhosis turn out to be NCIPH after liver biopsy (1,2)

NCIPH usually appears to have a benign course, however patients may worsen to a stage requiring liver transplantation(3). The exact etiopathogenesis of this common condition is still unknown. There are various hypotheses like infective, immunogenic, prothrombotic and trace elements exposure.

There are data to support the association between gut disorders, prothrombotic conditions and NCIPH. Eapen et al found 16% of NCIPH patients to have celiac disease and 3% to have ulcerative colitis(4). Hillaire et al found association between prothrombotic conditions like myeloproliferative disorders and NCIPH(5). Considering the above associations, we postulate a hypothesis that a combined effect of gut disorders and prothrombotic conditions lead to portal microangiopathy.

Gut disorders like celiac disease are commonly associated with altered intestinal permeability called as leaky gut(6). This leakiness can lead to migration of bacteria and bacterial products from the gut which is termed as bacterial translocation(7). In the presence of a preexisting prothrombotic state in portal circulation, these gut factors could trigger a thrombotic event.

It is well known that in liver cirrhosis, altered intestinal permeability and bacterial translocation happen, particularly in patients with ascites and advanced cirrhosis(8).

Bacterial translocation has been proposed to play a role in progression of liver fibrosis(9) However there is no data on association between NCIPH and bacterial translocation.

ADAMTS13 (a disintegrin and metalloprotease with a thrombospondin type 1 motif, member 13) is a protease, action of which is to cleave von Willebrand factor multimers released from activated/ damaged endothelium. If there is a deficiency in the ADAMTS13 activity, ultralarge vWF accumulate and cause platelet aggregation under shear stress that results in microangiopathy called thrombotic thrombocytopenic purpura (TTP). ADAMTS13 is exclusively synthesized from stellate cells, so the portal blood is expected to have the lowest concentration due to its upstream relationship to stellate cells(10).

It is documented that in liver cirrhosis, with advancing liver disease ADAMTS13 activity comes down proportionately along with a corresponding increase in vWF activity and multimers(11). Mackie et al analyzed the ADAMS13 activity in NCIPH patients and found that irrespective of the severity of liver dysfunction, NCIPH patients have low ADAMTS13 activity(12)

Based on the above literature we designed our hypothesis as follows: A combination of gut disorders which make the gut leaky for gut derived factors to enter circulation and the preexisting ADAMTS13 deficiency, act together in the pathogenesis of portal microangiopathy.

AIM OF THE STUDY

Hypothesis:

A combination of gut disorders which make the gut leaky for gut derived factors to enter the portal circulation and a preexistent prothrombotic state in portal circulation due to ADAMTS13 deficiency, act together to produce obliterative portal microangiopathy in Non cirrhotic intrahepatic portal hypertension (NCIPH)

Aim of the study:

Aim 1- To study the association between gut disorders, bacterial translocation and NCIPH.

Aim 2- To assess the status of ADAMTS13 (a disintegrin and metalloprotease with a thrombospondin type 1 motif, member 13) in NCIPH patients and compare with that of controls.

REVIEW OF LITERATURE

Non Cirrhotic Portal Hypertension means portal hypertension in the absence of cirrhosis. This can be either intrahepatic or extrahepatic. Non cirrhotic Intrahepatic Portal Hypertension (NCIPH) is a broad terminology encompassing multiple entities characterized by presence of intrahepatic portal hypertension without cirrhosis(13). These entities include Non cirrhotic portal fibrosis (NCPF), Idiopathic portal hypertension (IPH), Nodular regenerative hyperplasia (NRH), partial nodular transformation, incomplete septal sclerosis, hepatoportal sclerosis and benign intrahepatic portal hypertension.

Asian Pacific Association for the Study of the Liver (APASL), Working Party on Portal Hypertension defined NCIPH as

"A disease of uncertain etiology, characterized by periportal fibrosis and involvement of small and medium branches of the portal vein; resulting in the development of portal hypertension. The liver functions and structure primarily remain normal."(14)

Historical background:

In early 1960s, various researchers from many parts of the world recognized the presence of obliterative portal venopathy in patients with non cirrhotic disease presenting with bleeding varices. (15–17) Since then the understanding of the disease improved significantly. This distinct clinico-pathological entity was termed as noncirrhotic portal fibrosis (NCPF) in India and as idiopathic portal hypertension (IPH) in Japan. Several

different terminologies have been described; these probably represent the varied spectrum of the same disease.

Epidemiology:

NCIPH has been reported to clinically manifest in 3rd to 4th decade of life. Reports from India have shown either no gender predilection or a male predominance, where as reports from Japan showed female preponderance. Mainly the disease affects socioeconomically disadvantaged people(18) This may explain the decreasing incidence of this entity in developed countries.

However it is still a commonly encountered clinical condition in developing countries. Studies from our centre document NCIPH to be a common cause for cryptogenic intrahepatic portal hypertension. Retrospective analysis of portal hypertensive patients who underwent liver biopsy, showed that 48%(30/62) of patients labeled as cryptogenic cirrhosis turned out to have NCIPH after liver biopsy(1) Prospective analysis of 610 portal hypertensive patients showed cryptogenic chronic liver disease to be the commonest cause of portal hypertension. Among the cryptogenic patients who underwent liver biopsy, 39% had NCIPH , not the true cryptogenic cirrhosis(2). Thus the prevalence of NCIPH was 39 to 48% among the patients who underwent liver biopsy with a clinical diagnosis of cryptogenic cirrhosis.

Clinical presentation and Natural history:

Clinical presentations are mainly related to portal hypertension, either bleeding or non bleeding varices, splenomegaly or hypersplenism. Liver function remains well preserved for long time. Majority of patients tend to have pancytopenia particularly thrombocytopenia. Liver may appear normal or nodular in ultrasonography. Esophageal or gastric varices are noted in >95% of patients. Hepatic venous pressure gradient can be normal or slightly elevated.

Generally NCIPH patients have good prognosis(19,20). However the disease can progress to a stage requiring liver transplantation. This has been convincingly demonstrated by study of explant livers from clinically cirrhotic individuals(3) Eapen et al reported progression to liver failure in 53% of NCIPH patients over a median follow up period of 88 months, indicating that NCIPH is not always a benign disease(4)

Pathophysiology:

The histological hallmark of NCIPH is obliterative portal venopathy as termed by Nayak and Ramalingaswami in 1969(21) The denominator for the varied morphological changes noticed in NCIPH is the occlusive portal microangiopathy(5). There is portal and periportal fibrosis which lead to alteration in intrahepatic portal blood flow. Aberrant ectatic vessels can be seen representing a process similar to collateral formation(22). Above changes are typically patchy. Subcapsular atrophy and nodular hypertrophy happen due to the patchy insufficiency in portal supply(23). Long standing insufficiency results in phlebosclerosis of hepatic vein branches(24).

Liver biopsy and documentation of absence of advanced fibrosis or cirrhosis is the most important requirement to differentiate NCIPH from cirrhosis.

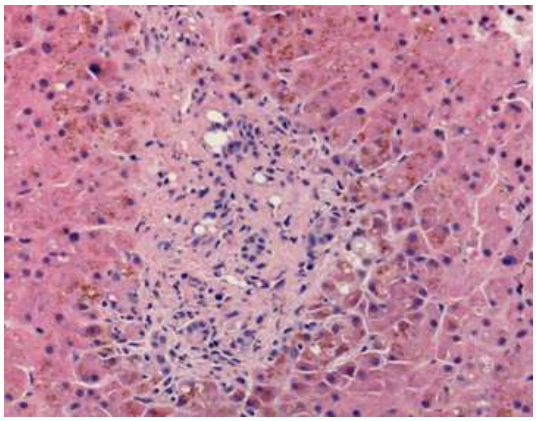


Figure 1: Densely fibrotic portal tract with occlusion of portal vein

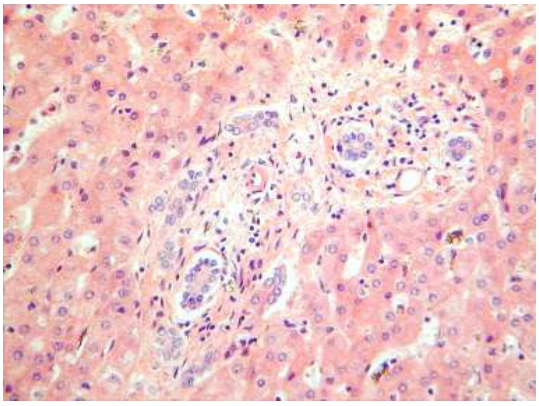


Figure 3: Two abnormally opposed portal tracts without portal veins

Figure 2: Ectatic portal vein branch

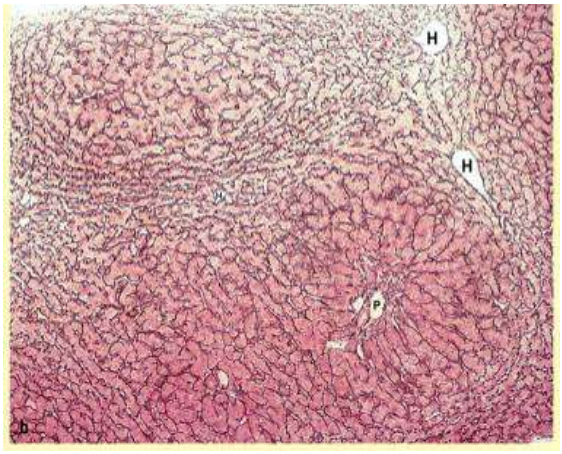


Figure 4: Nodular regenerative hyperplasia

Etiopathogenesis:

Etiology is unknown. Many different mechanisms like Infective, Immunogenic, Prothrombotic and exposure to trace chemicals are proposed.

Infective hypothesis:

Since NCIPH is a disease prevalent in developing countries among low socio economic population, infective hypothesis is postulated. Repeated diarrheal disease, neonatal umbilical sepsis or other intraabdominal infections are likely to result in portal pyemia which in turn can lead to portal pylephlebitis, thrombosis, sclerosis and obstruction of small portal vein branches(25). This hypothesis is supported by animal models , where NCIPH like pathology had been demonstrated after intraportal injection of nonpathogenic E.coli(26)

Immunogenic hypothesis:

There is some evidence that there may be deranged immune response or autoimmunity playing role in NCIPH(27)

Exposure to trace chemicals:

Chronic arsenic, vinyl chloride exposures have been described to be associated with development of NCPF(28,29) Other factors like hypervitaminosis A, chronic exposure to azathioprine, methotrexate, 6-mercaptopurine, didanosine have also been observed to have relation with NCIPH.

Hypercoagulability:

Hillaire et al reported strong association of prothrombotic conditions with NCIPH. In this study extensive prothrombotic work up was done in 23 patients, 12 out of 23(48%) were found to have associated one or more prothrombotic conditions- five

patients had myeloproliferative disorder, four had protein S deficiency, one had protein C deficiency, five had high anticardiolipin antibody. On long term follow up 13 patients developed extrahepatic portal venous thrombosis supporting the existence of prothrombotic status. The authors concluded that anticoagulant therapy is to be strongly considered in NCIPH patients(5).

In spite of many hypothesis and supporting evidence, the exact etiology and pathogenesis of changes in NCIPH are not yet known, due to which the management of patients with NCIPH is only symptomatic and supportive.

Researchers have found association of NCIPH with gut diseases as well as various prothrombotic conditions. These concepts led to derivation of new hypothesis for the etiopathogenesis of the entity.

Gut disorders and NCIPH:

Various studies have documented possible association between NCIPH and gut disorders, particularly celiac disease. In 2006, Sharma et al from India reported two cases of celiac disease with coexistent NCPF, and they postulated autoimmune mechanisms to drive the pathogenesis of the two conditions together(30). Similar association of celiac disease with Nodular regenerative hyperplasia had also been demonstrated(31,32) Zamani et al from Iran reported a case of decompensated NCIPH associated with celiac disease complicated with ulcerative jejunoileitis and intestinal lymphoma(33)

A search for the coexistent gut disorders among 34 NCIPH patients was made by Eapen at al(4). Among 31 patients tested for celiac serology by IgA anti tissue transglutaminase/ anti endomysial antibodies, five (16%) tested positive for celiac

serology and also had duodenal histology consistent with celiac disease. Three patients (9%) had ulcerative colitis.

Data from our centre support the high prevalence of celiac disease among cryptogenic chronic liver disease patients (22% - 11 out of 51). Seven patients out of these eleven underwent liver biopsy and five of them were found to have NCIPH.

Since the pathology in NCIPH is primarily confined to the portal circulation characterized by selective portal microcirculatory obliteration, causative factor may be derived from the gut since the portal circulation forms the first filter for gut derived factors. In the presence of gut disorders, there is a potential for various gut derived factors like bacteria, bacterial products, inflammatory cytokines, immunoglobulins etc. to enter portal circulation.

Prothrombotic conditions and NCIPH:

As mentioned earlier, various hypercoagulable conditions have been found to be coexisting in NCIPH patients(5) Eapen et al demonstrated elevated anticardiolipinA antibody among patients with NCIPH when compared to patients with Budd- Chiari syndrome. Elevation in anticardiolipinA antibody was found to be significantly higher than that of anticardiolipinG and anticardiolipinM antibodies. IgA antibodies form the candidate gut derived antibodies, hence the authors postulated that the prothrombotic factors in NCIPH are probably gut derived and affect the portal circulation which is the first filter for gut derived factors(4).

Elevated IgA anticardiolipin antibodies in patients with celiac disease and nodular regenerative hyperplasia has been described by many authors(31,32)

Selective obliteration of portal microcirculation may be either due to gut derived prothrombotic factors entering the portal system culminating in the thrombotic event or a preexistent prothrombotic state in portal circulation with gut derived factors triggering the thrombotic event.

Mackie et al hypothesized that an imbalance in von Willebrand factor (VWF) and ADAMTS13 (a disintegrin and metalloprotease with a thrombospondin type 1 motif, member 13) which is observed in Thrombotic thrombocytopenic purpura(TTP), if confined to portal circulation at the point where hepatic arterial pressures are superimposed, would provide a mechanism for development of portal microangiopathy similar to systemic microangiopathy occurring in TTP(12).

In this study, 18 NCIPH patients and 25 controls with chronic liver disease of other etiologies were included. Cases and controls were comparable with regard to the severity of liver disease. ADAMTS13 activity assay, ADAMTS13 antigen levels, ADAMTS13 antibody levels, VWF antigen, activity and VWF multimer levels were measured.

It was observed that ADAMTS13 activity and antigen levels were significantly reduced in cases when compared to controls. Marked reduction in activity (activity <5%) was noted in 28% of cases and in none among the controls. Corresponding reduction in antigen levels was also observed. Notably most of the NCIPH patients had low platelet count which was disproportionate to MELD score unlike the disease controls, which

indicates that they may be additional mechanisms for thrombocytopenia in NCIPH patients. The study looked for possible presence of ADAMTS13 antibodies/inhibitors as the cause for low activity, however there was no difference between the cases and controls and there was no correlation between the presence of antibodies and low activity.

One third of the patients had ultra large VWF multimers. VWF Ag and activity were either normal or increased in both cases and controls. There was a correlation between VWF activity and MELD in both cases and controls.

Sustained low ADAMTS13 activity was noticed in four NCIPH patients over a period of six weeks to eleven months.

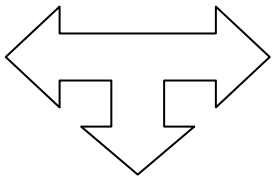
Authors concluded sustained ADAMTS13 deficiency to be characteristic in NCIPH irrespective of severity of liver disease. They proposed abnormally unresponsive hepatic stellate cells as the probable reason for ADAMTS13 deficiency and absence of hepatic fibrosis.

Based on the above literature on gut disorders and prothrombotic conditions among NCIPH patients, we made the following hypothesis.

Hypothesis

Presence of gut disorders which make the gut leaky for gut derived factors to enter the portal circulation

Existence of prothrombotic state in portal circulation due to ADAMTS13 deficiency



Obliterative portal microangiopathy

Leaky gut:

The gastrointestinal tract apart from serving its primary functions of digestion and absorption plays a major role as a barrier between environment and host. The intestinal barrier has mechanical and functional components. The mechanical barrier is formed by the intestinal epithelial layer particularly the tight junctions between the epithelial cells, mucus coat on the epithelial surface, sub epithelial connective tissue and capillary endothelium. Functional barrier is formed by specialized epithelial cells like paneth cells, gut associated lymphoid tissue and its immune cells(34,35)

The tight junctions are complex and dynamic; they are selectively permeable to micromolecules and impermeable to bacteria and macromolecules like lipopolysaccharides. They are regulated by variety of stimuli like dietary factors, neuronal and humoral stimuli and inflammatory mediators. Gut microbiota play an important role in maintaining the integrity of this barrier function.(36) Intestinal permeability can be affected by various factors like reactive oxygen species produced as a result of reperfusion injury or intestinal inflammation, inflammatory mediators, nitric oxide overproduction, Interleukin 6,certain bacteria like E.coli and Klebsiella, alcohol, drugs like NSAID etc(7)

Leaky gut describes hyperpermeability of intestinal epithelial barrier. Such leakiness ultimately results in bacterial translocation which means translocation of bacteria or bacterial products across the intestinal barrier(7)

It is well documented that gut disorders like inflammatory bowel disease, celiac disease, tropical spure can be associated with leaky gut(6,37,38),hence bacterial translocation can happen in these conditions(39),(40)

Measures of bacterial translocation:

Identification of intestinal bacteria in mesenteric lymph nodes (MLNs) is the direct evidence of bacterial translocation (BT), however sampling MLNs is difficult. Demonstration of intestinal bacteria or its products like endotoxin in portal/ peripheral blood is an indirect evidence of BT. Bacterial identification by PCR method is superior to culture method(41–43) Amplification of a region of 16s rRNA gene, a conserved eubacterial gene is a sensitive method for detection of bacteria(44)

Though bacterial translocation can occur in the absence of altered intestinal permeability(40), leaky gut is a trigger for bacterial translocation(7,45) Therefore measurement of IP(intestinal permeability) can also serve as an indirect measure of bacterial translocation.

Loss of intestinal integrity can be assessed by various methods(46). Most commonly used among those is differential sugar absorption test (DST). This test is based on the fact that larger oligosaccharides like lactulose do not permeate through intact epithelial barrier, whereas smaller monosaccharides do so. On simultaneous oral administration of a large and a small molecular probe, both will enter the circulation according to the permeability status following which they will be excreted in urine. Measurement of the ratio of recovery in the urine will then give information about the paracellular passage. Usage of two compounds and measurement of ratio of recovery help to overcome confounding factors like gastric emptying, bacterial degradation, gastric dilution, intestinal transit and renal function(36,46) The differential sugar absorption test using Lactulose and Mannitol is found to have high sensitivity and specificity in

assessing small intestinal permeability(47) Other tests used to assess IP are polyethyl glycol permeation study and chromium labeled EDTA permeation study.

Clinical relevance of bacterial translocation (BT):

Bacterial translocation happens when there is damage to intestinal barrier or when there is immune suppression. Berg stated that a baseline translocation in normal physiological state may be a boost to the immune system(34) Much evidence exits to support bacterial translocation as the cause for infectious complications in critically ill patients like trauma, burns, postoperative patients and in patients with multi organ dysfunction(48) Spontaneous bacterial peritonitis occurring in cirrhotics is due to intestinal bacterial translocation. Selective intestinal decontamination is helpful in preventing infectious complications in cirrhotic patients with gastrointestinal hemorrhage and low protein ascites. Such measure also prevents SBP recurrence(49)

Bacterial translocation and cirrhosis:

There is lots of research conducted with regard to bacterial translocation in cirrhosis. Animal studies have shown that detection of bacterial DNA in peripheral fluids like ascitic fluid or pleural fluid or blood represent intestinal bacterial translocation(50). Studies have shown that around one third of patients with cirrhosis and non neutrocytic culture negative ascites have detectable peripheral blood and ascitic fluid bacterial DNA(44,51),(52) Its well recognized that the bacterial translocation occurs predominantly in advanced cirrhosis. In a study by Bellot et al, peripheral blood bacterial

DNA was positive in nearly 40% of cirrhotic patients with ascites, but in none among those without ascites(8)

Mechanisms of bacterial translocation in cirrhosis:

Intestinal hypomotility, stasis and bacterial overgrowth, altered intestinal permeability due to oxidative stress at intestinal mucosal level, nitric oxide overproduction leading to leaky gut, poor immune response in MLN are all the possible mechanisms for bacterial translocation in cirrhosis(49)

Scarpellini et al observed a significant association between the advanced liver disease and altered intestinal permeability, a direct correlation was noted between worsening liver cirrhosis as per child pugh classification and the prevalence of bacterial translocation among cirrhotics. 75% of child's C patients, 39% of child's B patients and 22% of child's A patients were found to have impaired IP(45).

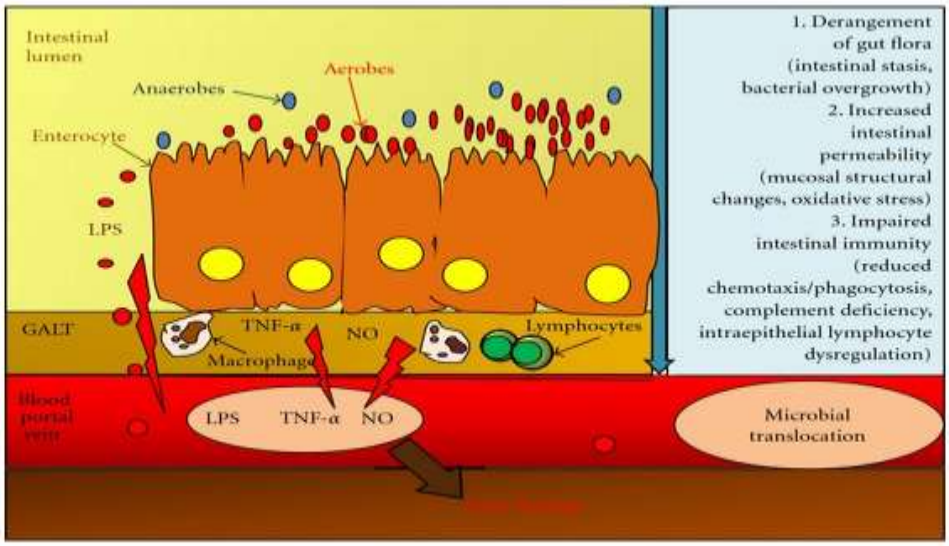


Figure 5: Mechanisms of bacterial translocation in liver cirrhosis

Consequences of bacterial translocation in cirrhosis:

Bacterial translocation is considered to be the prime mechanism for infectious complications, particularly SBP in cirrhotic patients. Presence of peripheral blood bacterial DNA is associated with increased nitric oxide production, peripheral vasodilatation, systemic circulatory dysfunction, intrahepatic endothelial dysfunction and high levels of proinflammatory cytokines like TNF alpha(8) These changes increase the risk of development of hepatorenal syndrome and SBP, ultimately increasing the risk of death(53). Bacterial translocation as evident by positive serum and ascitic fluid bacterial DNA has been observed to be an independent prognostic marker in non infected patients with advanced cirrhosis(52)

Role of bacterial translocation in liver fibrosis:

There are recent advanced studies demonstrating the role of Toll like receptors (TLRs) in the liver fibrogenesis. Hepatic stellate cells are the prime cell types in liver fibrogenesis, they get transformed to myofibroblasts upon stimulation by factors like TGFβ and PDGF which are released from the adjacent kupffer cells. In liver disease patients, altered intestinal permeability and bacterial translocation can activate the TLRs of kupffer cells, endothelial cells and stellate cells as a part of innate immune response. This results in potent immune response and activation of stellate cells(9,54) Thus bacterial translocation is important in progression of liver fibrosis.

Leaky gut and gut derived bacterial products are considered to be of fundamental importance in the pathogenesis of Alcoholic liver disease (55) and in Non alcoholic fatty liver disease(56)

Bacterial translocation and NCIPH:

As stated above, NCIPH is found to be associated with various gut disorders and such gut diseases can be associated with leaky gut and bacterial translocation. Nevertheless, the role of leaky gut and bacterial translocation in the pathogenesis of NCIPH has not been studied so far.

ADAMTS13: (a disintegrin and metalloprotease with a thrombospondin type 1 motif, member 13)

ADAMTS13 is a metalloprotease, the function of which is to cleave von Willebrand factor (VWF) multimers. Congenital or immune mediated deficiency in ADAMTS13 activity, reduce or abolish the cleavage of unusually large VWF multimers. Ultralarge VWF released from activated endothelial cells would accumulate if the protease activity is subnormal. This leads to platelet clumping and thrombi formation under high shear stress resulting in microangiopathy characterized by schistocytic hemolysis, thrombocytopenia and multiorgan microcirculatory disturbances.

Thrombotic thrombocytopenia purpura (TTP) is a condition characterized by thrombotic microangiopathy due to deficient ADAMTS13. Hereditary TTP is due to homozygous/ heterozygous mutation in ADAMTS13 gene whereas acquired TTP is due

to antibody formation. Even in hereditary TTP, clinical manifestations due to microcirculatory disturbance occur only in the presence of triggering factors such as pregnancy, infection, drugs, malignancy. Treatment of TTP is plasma exchange with FFP which replenishes ADAMTS13(57).

ADAMTS13 deficiency can occur in varied clinical situations such as acute inflammatory conditions like DIC, post operative period, uremia, liver cirrhosis, other thrombocytopenic conditions, late pregnancy, neonatal period, elderly age(58,59). However, severe ADAMTS13 deficiency defined as ADAMTS13 activity of <5% of normal is considered to be specific for TTP(60)

ADAMTS13 is produced almost exclusively by hepatic stellate cells(10), there is high chance that its level of activity will be affected in liver diseases.

ADAMTS13 and cirrhosis:

Advancing liver cirrhosis is associated with progressively increasing risk of platelet thrombi formation. This is due to the excess production of VWF multimers by damaged/ activated sinusoidal endothelial cells during liver injury, which in turn promote platelet aggregation. It has been documented that the levels of VWF antigen and activity are elevated in liver cirrhosis and the elevation is directly proportionate to the severity of the liver disease. The progressive increase in ultralarge VWF multimer is not only due to overproduction, but also due to the corresponding reduction in the ADAMTS13 activity noted in cirrhosis(11)

Possible mechanisms for reducing ADAMTS13 activity in cirrhosis are excess consumption by large quantities of VWF Ag, inhibition of activity by inflammatory cytokines or other inhibitors/ antibodies, decreased production from the stellate cells(61)

Uemura et al performed a comprehensive analysis of ADAMTS13 activity, antigen, VWF activity and multimer levels in 33 patients with chronic hepatitis and 109 patients with liver cirrhosis. They found that the mean ADAMTS13 activity was progressively decreasing with increasing severity of liver disease. In their study group, the mean ADAMTS13 activity was 87% in chronic hepatitis, 79% in Child's A cirrhosis, 63% in Child's B cirrhosis, and 31% in Child's C cirrhosis. Severe deficiency (<3% of controls) was noted in five end-stage cirrhosis patients. Plasma inhibitor for ADAMTS13 was detected in 83% of patients with moderate or severe deficiency.

The abnormally high VWF Ag: ADAMTS13 activity ratio can cause sinusoidal microcirculatory disturbances which lead to progressive liver injury and also contribute to multiorgan failure. This phenomenon has been documented in severe alcoholic hepatitis(62) and in graft dysfunction following liver transplantation(63)

Thrombocytopenia observed in advanced liver cirrhosis is multifactorial, contributing factors include hypersplenism, low thrombopoietin levels, myelosuppression of platelet production by HCV, alcohol. It is also proposed that high levels of Ultralarge VWF and platelet aggregation additionally contribute to thrombocytopenia(61)

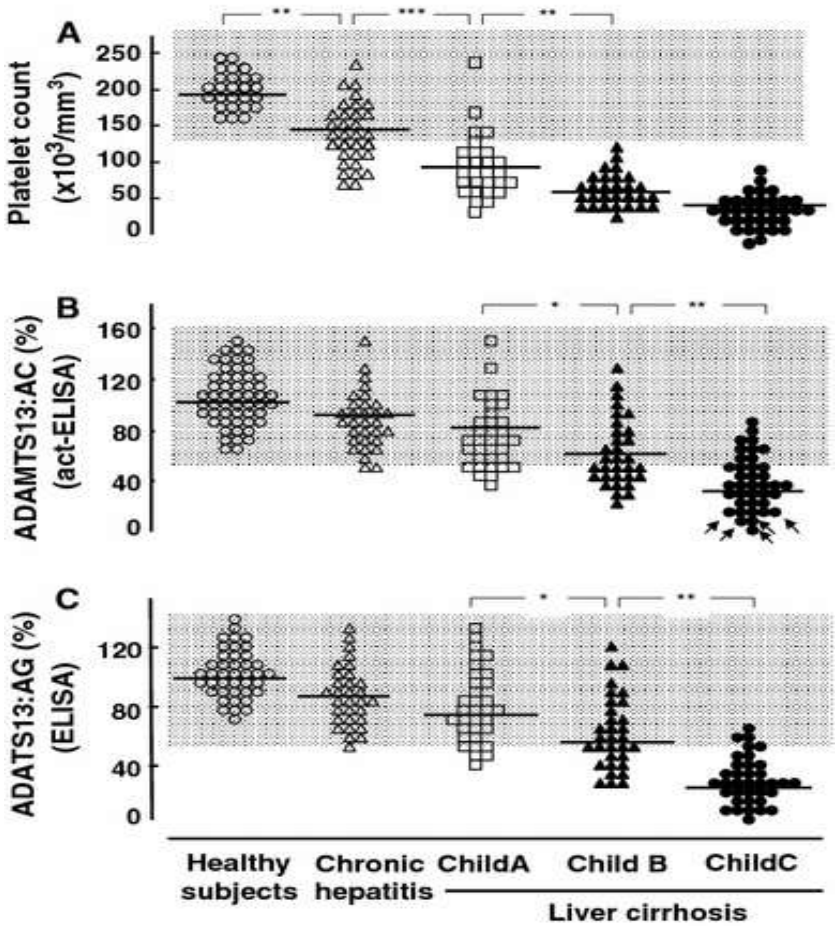


Figure 6: Platelet levels, ADAMTS13 activity and ADAMS13 antigen levels progressively decrease with worsening liver disease(11)

Recently Ferlitsch at al found VWF Ag level as a good predictor of presence of clinically significant portal hypertension, development of decompensation and mortality in liver cirrhosis(64)

ADAMTS13 and NCIPH:

Irrespective of the severity of liver dysfunction, low ADAMTS13 activity was noted in 18 NCIPH patients by Mackie et al (12). Severe deficiency which is considered to be specific for TTP was also observed in 28% of NCIPH patients.

NCIPH is invariably associated with thrombocytopenia which is often attributed to hypersplenism, but it can be disproportionate to hypersplenism, hence there is a possibility that additional mechanisms exist. Development of portal microangiopathy in NCIPH may represent a “localised TTP” happening at the portal microcirculation due to low ADAMTS13 activity.

METHODOLOGY

Study Design: Case control study

Study Period: March 2011 to November 2012

Study Setting: Department of Gastrointestinal sciences,
Christian Medical College.

Study Subjects:

Cases: These were patients with liver biopsy proven Non Cirrhotic Intrahepatic Portal Hypertension (NCIPH)

Inclusion criteria: Patients who fulfilled all the following five criteria were included as cases.

1. Presence of portal hypertension as evidenced by any two or more of the following- varices, high SAAG ascites, hepatic venous pressure gradient more than 5 mm of Hg, cytopenia secondary to hypersplenism,
2. Patent portal and hepatic veins by Doppler ultrasound,
3. Liver biopsy consistent with NCIPH i.e. Absence of advanced fibrosis/ cirrhosis,
4. Exclusion of conditions causing chronic liver disease such as viral hepatitis, alcoholic hepatitis, metabolic causes like Wilson's/ Hemochromatosis, autoimmune hepatitis, Non alcoholic steatohepatitis,
5. Conditions that may cause NCIPH like lesions like congenital hepatic fibrosis, Sarcoidosis.

Exclusion criteria:

1. Patients with recent gastrointestinal bleed
2. Hepatocellular carcinoma
3. Active infections
4. Current antibiotic use

Controls: These were patients with Hepatitis B or Hepatitis C related chronic liver disease with portal hypertension. Diagnosis was made based on serology (a positive HBSAg or anti HCV antibody), imaging (Ultrasonography consistent with chronic liver disease) and upper GI scopy confirming the presence of varices. Exclusion criteria were same as that for cases.

Consent: Informed consent was taken from all the patients.

Study protocol was approved by Institutional Review Board (IRB)

Methods:

Demographic data:

Demographic data like age, gender, residence, socioeconomic status were collected from the study subjects.

Baseline investigations:

The following are the baseline investigations done in all study subjects.

1- Etiological workup for chronic liver disease (Viral serology for hepatitis B and C, if negative autoimmune profile that included ANA,SLA,LKM,SMA, Wilson's workup that included 24 hours urine copper and serum ceruloplasmin, Iron studies)

2- Ultrasonography of abdomen to look for liver size, liver space occupying lesion
3- Doppler to look for portal vein thrombosis, Budd chiari syndrome.
4- Upper GI scopy to assess varices
5- Ascitic fluid analysis if ascites was present
6- Liver biopsy, either percutaneous of transjugular route as indicated. NCIPH was diagnosed only in the absence of advanced fibrosis or cirrhosis.(biopsy was done only in cases not in controls)
7- Baseline biochemical investigations like liver function test and renal function test, Alpha fetoprotein, Prothrombin time with INR, Complete blood count.

Aim 1: To look for association between gut disorders, bacterial translocation and NCIPH.

Variables chosen:

1- Assessment of Celiac disease spectrum by testing serum IgA anti tissue transglutaminase antibody and duodenal histology.
2- Intestinal permeability assessment using urinary Lactulose/ Mannitol ratio (an indirect measure of bacterial translocation)
3- Peripheral blood bacterial DNA quantification by detection of 16S rRNA gene using PCR.(a marker of bacterial translocation)
4- Plasma Tumor necrosis factor (TNF) alpha levels (a measure of consequence of bacterial translocation)

Aim 2: To assess the status of ADAMTS13 (a disintegrin and metalloprotease with a thrombospondin type 1 motif, member 13) in NCIPH patients and compare with that of controls.

Variables chosen:

1- Plasma ADAMTS13 activity assay

2- Plasma ADAMTS13 antigen estimation

Celiac disease spectrum:

Anti tTG antibody: Evaluation of IgA antibody against neo epitopes of tissue transglutaminase was performed using commercially available solid phase enzyme immuno assay kit (AESKULISA Celichek, Germany and AIDA, Germany) The assay employed recombinant human transglutaminase. Quantitative interpretation of patient's sample was obtained in U/ml, titers < 15 U/ml was taken as negative, 15 to 20 as borderline and >20 as positive.

Duodenal histology: Upper gastrointestinal endoscopy was done and duodenal biopsy was taken from D2. Celiac enteropathy grading was done based on Marsh grading.

Intestinal permeability testing by urinary L/M ratio:

After an overnight fast the patient emptied his or her bladder and drank a solution containing 2 g mannitol and 5 g lactulose made up to 100 ml with demineralised water. For the first 2 hours after drinking the test fluid, no food or fluid was allowed and all the urine passed in the 5 hours after they had drunk the test fluid was collected. Chlorohexidine digluconate 20% (0.5 ml) was added to the urine as a preservative. The

urine volume was measured and aliquots were stored frozen at -20°C. To calculate the lactulose/mannitol ratio, samples were analyzed for lactulose and mannitol by gas chromatography. A lactulose/mannitol ratio ≥0.086 was considered abnormal.

Plasma TNF alpha levels:

TNF alpha estimation was done in stored plasma samples using capture ELISA method (BD OptEIA™ -BD biosciences, San Diego)

Peripheral blood bacterial DNA quantification:

Whole blood sample was collected under aseptic precautions into sterile vacutainer tubes with EDTA. Plasma samples were centrifuged and DNA was extracted by salting method and the precipitate was stored in deep freezer at -20°C until further analysis.

Real time PCR:

Target gene was 16s rRNA gene, a conserved eubacterial gene. The primer set used was 3'-CGGTGAATACGTTCCCGG-5' and reverse primer 3'-TACGGCTACCTTGTTACGACTT-5'. Amplicon size for above mentioned primers was 145bp (Bioserve Biotechnologies, India). Master mix for PCR assay was from (Ref. No. RT-SY2X-03+WOUN) Eurogentec,Belgium. Chromo4 real time PCR detection system (Biorad, USA) was used for quantification. Total bacterial levels were expressed as CFU per ml of sample and calculated by plotting standard graph against bacterial plasmid DNA standards.

ADAMTS13 activity assay and antigen level measurement:

Citrated whole blood sample was collected in vacutainer tubes. Platelet poor plasma was separated by centrifugation and aliquoted and frozen at -80°C until assay. ADAMTS13 activity assay and antigen level measurement were done using peptide substrate chromogenic assay (Technozym® ADAMTS13 ELISA) Normal ADAMTS13 activity was taken as 60 to150% and normal ADAMTS13 antigen level was taken as 0.48 to1.59 µg/ml after standardization.

Statistical analysis:

Sample size: Study design was a case control study. With an assumption that 30% of NCIPH patients would have low ADAMTS13 activity and/ or positive peripheral blood bacterial DNA PCR and the same in controls would be 5%, we arrived at a sample size of 35 patients in each group with 80% power and an alpha error of 5%

Statistical Methods: Continuous variables were expressed in median and range. Man Witney U test was used for comparison of continuous variables. Fischer exact test was used to compare discrete variables. Spearman correlation coefficient was used for assessing correlation between variables. SPSS 15 software was used for analysis, a p value of <0.05 was considered significant.

Funding: Fluid research grant, Christian Medical College.

RESULTS

Total number of cases: 33 NCIPH patients

Total number of controls: 23 patients with Hepatitis B and 2 patients with Hepatitis C related chronic liver disease with portal hypertension.

Demographic data:

Median age of NCIPH patients was 36 years (range 21-62) and that of controls was 42 years (range 17-63). There were predominantly males in both the groups (21 out 33 cases and 23 out 25 controls were males).

Most of the patients in both the groups were in middle class by modified Kuppuswamy's socioeconomic status scale.

Table 1: Demographics

	Cases (n=33)	**Controls (n=25)**	**p value**
Age in years Median (range)	36 (21-62)	42 (17-63)	0.097
Gender (M:F)	21:12	23:2	0.015
Residence Northern India/ Southern India/Others	13/18/2	20/2/3	-
Socioeconomic status (Upper/Middle/Lower)	3/23/7	0/17/8	0.352

Baseline clinical characteristics:

Consecutive NCIPH patients were enrolled, whereas HBV/HCV related chronic liver disease patients were enrolled based on the Child's status and MELD score so that they matched with the cases. This was done due to the fact that the possibility of bacterial translocation increases with increasing severity of liver dysfunction(8,45); same is the case with reduction in ADAMTS13 activity(11). So it is essential that the two groups match each other in CTP status and MELD score.

Most of the patients in both the groups were in Child's A status (28 out of 33 cases and 20 out of 25 controls) There were no CTP C patients among cases and so no CTP C controls were taken(p value- 0.731) The median MELD score in NCIPH group was 10 with a range of 6 to 15. Among controls, the median MELD score was 10 with a range of 8 to 16(p value - 0.128)

Table 2: Clinical parameters

	Cases (n=33)	**Controls (n=25)**	**p value**
BMI (underweight/normal/ overweight/obese)	3/20/8/2	0/22/3/0	0.683
Child's status (A/B/C)	28/5/0	20/5/0	0.731
MELD score Median(range)	10 (6-15)	10 (8-16)	0.128
Platelet count x 10^3 /cu.mm Median (range)	62 (29-344)	72 (24-170)	0.85

BMI: Body Mass Index, MELD: Model for End Stage Liver Disease

There was thrombocytopenia noted in both cases and controls. The median platelet count was low in both the groups (62,000/cu.mm and 72,000/cu.mm respectively p-0.85). 23 out of 33 cases had platelet less than 1 lakh /cu.mm, totally six NCIPH patients had undergone splenectomy in the past, among them 3 had normal platelets; three had counts less than 1.5 lakhs.

Body mass index was noted to be preserved in most of the patients. Few of the NCIPH patients were obese. Three of the cases had diabetes mellitus.

Table 3: Clinical parameters

	Cases (n=33)	**Controls (n=25)**
Ascites Present/absent	5/28	4/21
Liver size Normal/shrunken	11/22	8/17
HVPG ≤5 mm of Hg/ >5 mm of Hg	7/8 (n-15)	nd
Variceal status (bleeder/nonbleeder/ novarices)	17/12/4	8/17/0

HVPG: Hepatic Venous Pressure Gradient, nd- Not done

Mild ascites was present in five cases and in four controls. None of them had past history of spontaneous bacterial peritonitis or large volume paracentesis. Two third of NCIPH patients had shrunken liver by ultrasonography.

Hepatic venous pressure gradient was measured in 15 NCIPH patients who underwent TJLB. Only 7 of them had low HVPG, rest had higher than 5 mm of Hg, the maximum HVPG was 15 in one patient.

17 out of 33 cases had past history of variceal bleed and that was the clinical presentation in most of them. 12 patients had varices without bleed, they were either incidentally detected to have varices during endoscopy or they presented with features of splenomegaly and hypersplenism. 4 did not have varices, clinical presentation in all of them was splenomegaly with or without hypersplenism. All the controls had varices as per the inclusion criteria. 8 of them had past history of variceal bleed, rest were non bleeders.

Serum Immunoglobulin levels were tested in 17 cases and 11 controls. IgG was more than upper limit of normal in 11 cases and 10 controls, IgA was more than limit of normal in 4 cases and 5 controls. None of the tested patients had immunoglobulin deficiency.

Celiac disease spectrum:

IgA anti tissue transglutaminase (tTG) antibody was tested in 32 NCIPH patients and in 10 controls.

NCIPH cases:

Nine cases were positive for the anti tTG antibody; seven of them were subjected to UGI scopy and D2 biopsy. Villous atrophy consistent with celiac disease was noted in 3 out of seven cases, three had non specific chronic duodenitis and the rest one had normal duodenal histology. Thus there were a total of 3 patients with celiac disease (positive serology and a consistent histology) among the NCIPH patients which accounts to 9.4%. There were 4 patients with latent celiac disease (positive serology with an inconsistent histology); the positivity may be due to false positive reaction as well which was not ruled out by doing IgA anti endomysial antibody.

Among the 23 cases, those who were negative for anti tTG antibody, duodenal histology was available for 8 patients. One of them had mild villous atrophy, four duodenal biopsies were normal; three were showing non specific duodenitis.

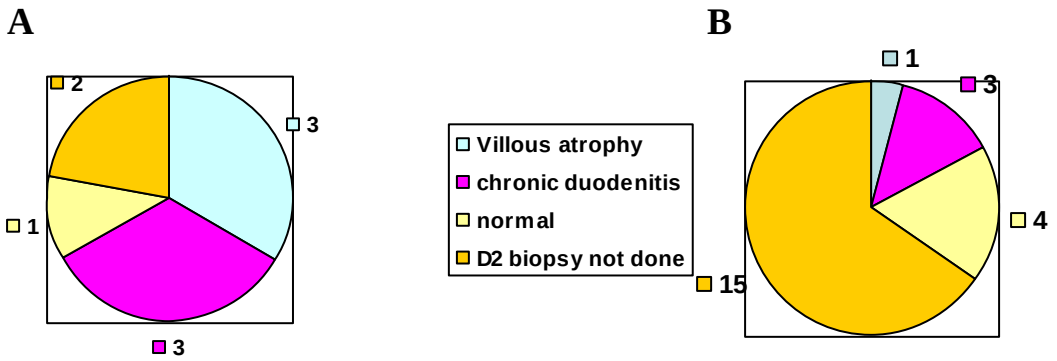


Figure 7: Duodenal histology A- anti tTG positive NCIPH patients and B- anti tTG negative NCIPH patients.

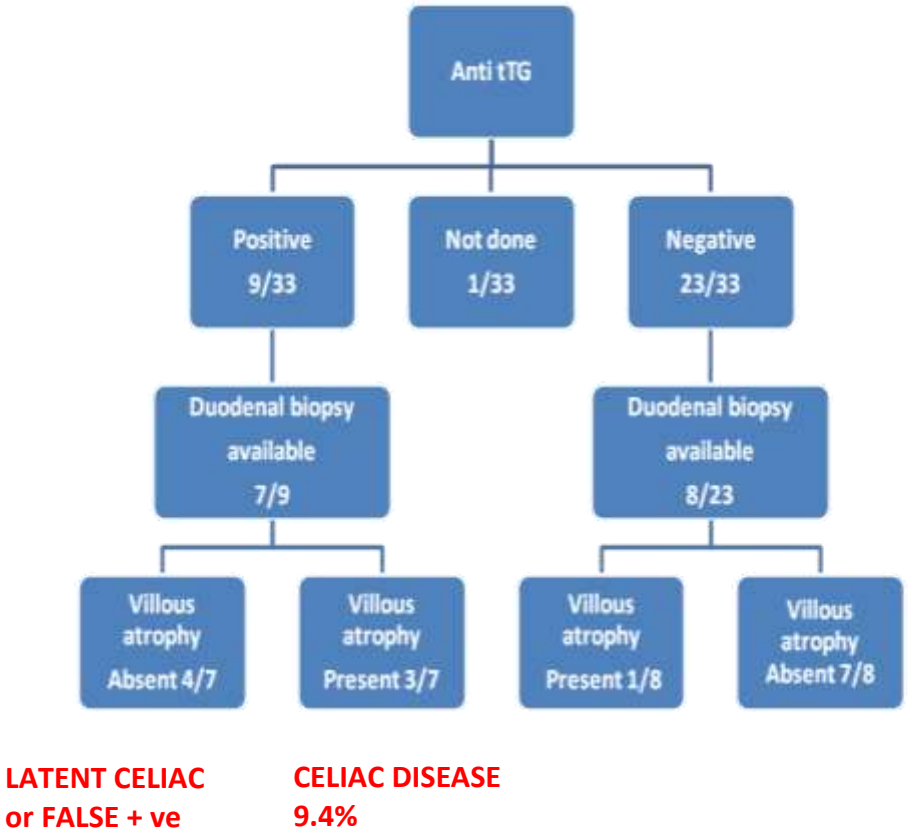


Figure 8: Celiac disease spectrum in NCIPH patients

Effect of gluten free diet:

All the three celiac disease patients were advised gluten free diet, anti tTG became negative on follow up for 2 patients, for the third patient, titer came down from 1412 IU/ml to 117.5 IU/ml. Two of the three latent celiac disease patients with duodenal biopsy showing chronic duodenitis were also on gluten free diet with which anti tTG antibody became negative in both of them.

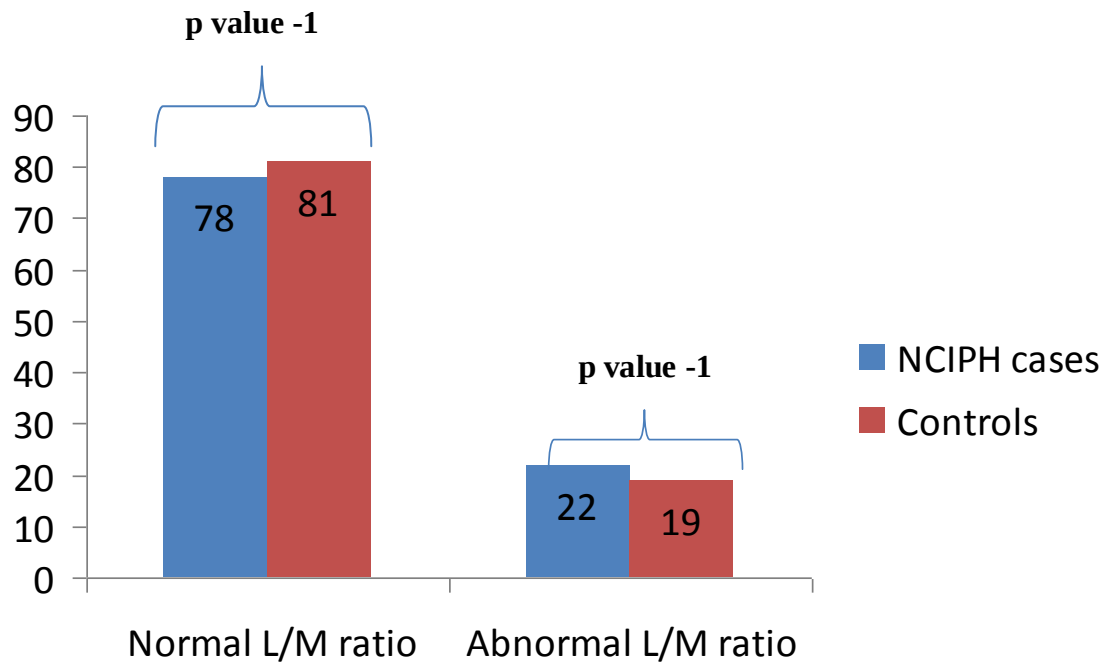
Study controls:

Out of the 10 controls tested for anti tTG antibody, three were positive for the Ab. However duodenal biopsy was not taken in these patients. Only one of these three patients had serum immunoglobulin tested and was having hypergammaglobulinemia.

Intestinal permeability testing by L/M ratio:

Assessment of intestinal permeability by dual sugar absorption test using Lactulose and Mannitol was performed in 26 cases (CTP A- 24, B-2) and 16 controls (CTP A-13, B- 3). In both the groups nearly 20% had abnormal intestinal permeability. The five NCIPH patients and three controls those who had abnormal IP were all in CTP A status and none of them had ascites. The test was invalid in three NCIPH cases due to coexistent glycosuria which made interpretation difficult.

Out of the three celiac disease patients, only one had L/M ratio tested and that was abnormal.



Tested in 26/33 NCIPH cases
Invalid result- 3
Normal- 18
Abnormal- 5

Tested in 16/25 Controls
Normal- 13
Abnormal- 3

Figure 9: Intestinal permeability in cases and controls

Table 4: Characteristics of patients with abnormal L/M ratio

		Cases (n=5)	Controls (n=3)
Child's status (A/B/C)		5/0/0	3/0/0
MELD score Median (range)		10(8-11)	11(9-11)
Anti tTG antibody (positive/ negative)		2/3	1/2
Duodenal histology	Not done/ normal	2	3
	Chronic duodenitis	2	0
	Villous atrophy	1	0

MELD: Model for End stage Liver Disease, tTG- tissue trans glutaminase

Peripheral blood bacterial DNA by PCR:

Due to technical difficulties PCR for detection of 16s rRNA gene could not be done. We encountered contamination and so all the samples including the no template controls (NTC) tested positive for bacterial DNA in unacceptably high colony forming units attributable to contamination. However, we could not find out the exact source of such bacterial contamination. So the test could not be standardized.

Plasma TNF alpha levels:

TNF alpha estimation was performed in plasma samples of 31 cases and 24 controls by ELISA method (BD OptEIA™). TNF α was not detectable in any of the samples.

ADAMTS13:

ADAMTS13 activity assay by chromogenic ELISA and estimation of ADAMTS13 antigen levels by ELISA was done in a group of cases and controls.

ADAMTS13 activity assay:

Assay was done in 20 NCIPH cases and in 13 controls. Most of them were in Child's A status, two in each group were in Child's B status. Both the groups were comparable with regard to the Child's status and MELD score.

Table 5: Clinical parameters of patients tested for ADAMTS13 activity

	Cases (n=20)	**Controls (n=13)**
Child's status (A/B/C)	18/2/0	11/2/0
MELD score Median (range)	9(7-13)	11(8-16)
Platelet count* x 10^3 /cu.mm Median (range)	61 (29-255)	61 (24-160)

* Three patients were post splenectomy patients.

Results:

Normal range of ADAMTS activity is 60 -150% for our lab. Activity less than 60% is low, if it is less than 5% it is taken as severely low.

Significantly low ADAMTS13 activity in cases than controls:

15 out of 20 cases were found to have low ADAMTS13 activity(75% of cases) , rest 5 had normal activity(25% of cases).Whereas among controls, only 4 had low activity (31%) and the rest 9 had normal activity(69%){ Figure-10}

Table 6: ADAMTS13 activity in cases and controls

Variable		Cases (n=20)	Controls (n=13)	p value
ADAMTS 13 activity (% of normal) Median (range)		44 (2-104)	104 (2-104)	0.04
ADAMTS 13 activity in Child's A patients Median(range)		42.9 (2-104) n= 18	104 (2-104) n= 11	0.02
ADAMTS 13 activity	<20%	6 (30%)	3 (23.1%)	0.03
	20-39%	1 (5%)	0	
	40-59%	8 (40%)	1 (7.7%)	
	60-150%	5 (25%)	9 (69.2%)	

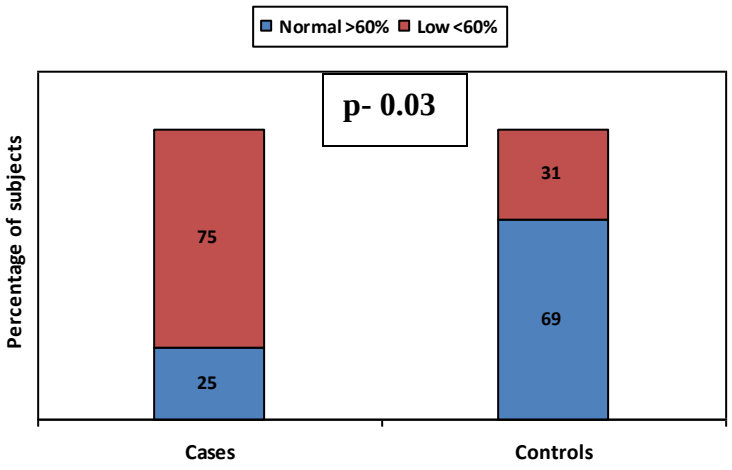


Figure 10: ADAMTS13 activity in cases and controls

Median ADAMTS13 activity among cases was 44% with a range of 2 to 104%, Median ADAMTS13 activity among controls was 104% with a range of 2 to 104%. The difference was statistically significant (p value- 0.04) (Figure 11}

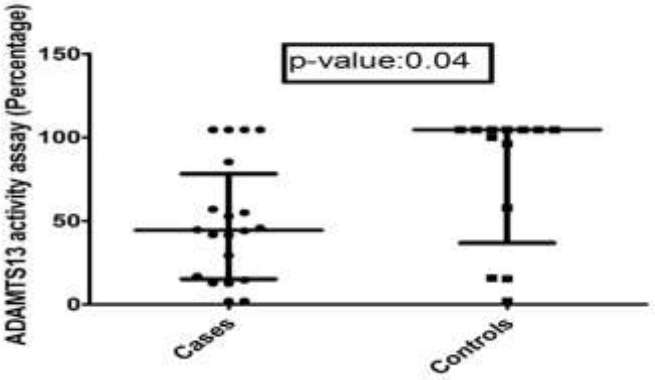


Figure 11: ADAMTS13 activity in cases and controls

Distribution of low activity among cases was in such a way that nearly half of them had mild reduction in activity – 8 patients out the 15 had activity in the range of 40 to 60%. On the other hand 6 patients had very low activity of <20%, 2 among them had severely low activity of <2%. Both the patients with severely low activity were in Child's A status. {Figure 12}

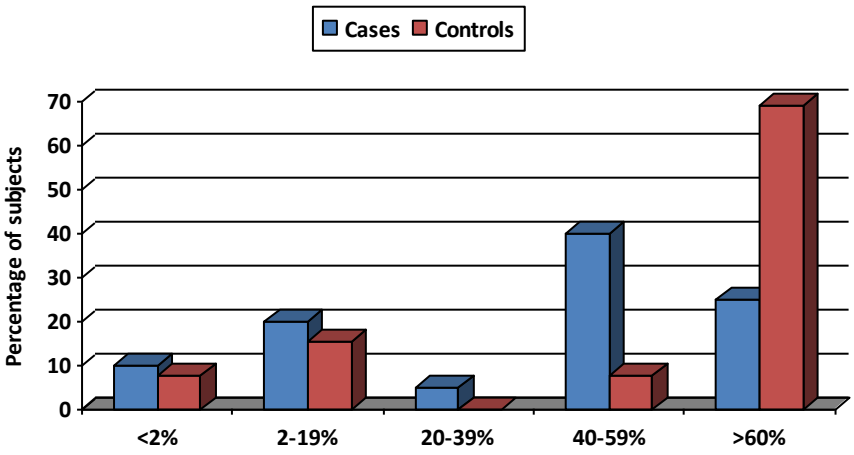


Figure 12: Distribution of ADAMTS13 activity in cases and controls

Significantly low ADAMTS13 activity in Child's A NCIPH patients vs. Child's A controls

Its well documented in literature that with advancing severity of liver dysfunction, plasma ADAMTS13 activity will decrease proportionately in cirrhotic patients(11)which implies that patients with early liver disease i.e. those in Child's A status are expected to have normal or only marginal reduction in ADAMTS activity.

We analyzed the data for Child's A patients alone and we found the ADAMTS13 activity to be significantly low among the cases when compared to controls.

Median ADAMTS13 activity among cases (n=18) was 42.9% with a range of 2 to 104%. Median ADAMTS13 activity among controls (n=11) was 104% with a range of 2 to 104%. The difference was statistically significant (p value- 0.02) (Figure 13}

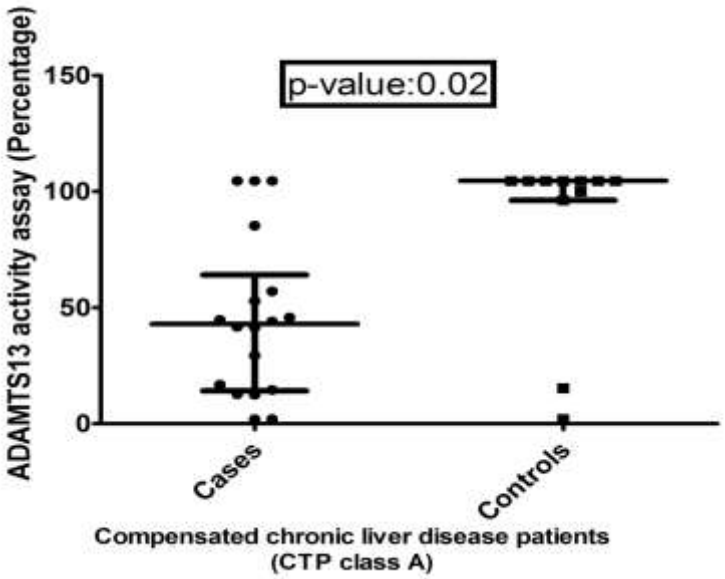


Figure 13: ADAMTS13 activity in cases and controls with Child's A liver disease

Among the CTP A patients, 14 out of 18 cases had low ADAMTS13 activity, whereas only 2 out of 11 controls had low activity (77.8% vs. 18.2% p value- 0.005)

ADAMTS13 antigen assay:

Assay was done in 25 NCIPH cases and 16 controls. Most of them were in Child's A status, four in each group were in Child's B status. Both the groups were comparable with regard to the Child's status and MELD score.

Table 7: Clinical parameters of patients tested for ADAMTS13 antigen

	Cases (n=25)	**Controls (n=16)**
Child's status (A/B/C)	21/4/0	12/4/0
MELD score Median (range)	10(6-15)	11(8-16)
Platelet count* x 10^3 /cu.mm Median (range)	62 (29-344)	61 (24-160)

* Five patients were post splenectomy patients.

Results:

Normal range of ADAMTS antigen level is 0.48 to1.59 µg/ml for our lab.

Significantly low ADAMTS13 antigen level in cases than controls:

23 out of 25 cases were found to have low ADAMTS13 antigen(92% of cases) , only 2 had normal antigen(8% of cases).Whereas among controls, only 7 had low antigen(44%) and the rest 9 had normal antigen(56%){ Figure-14}

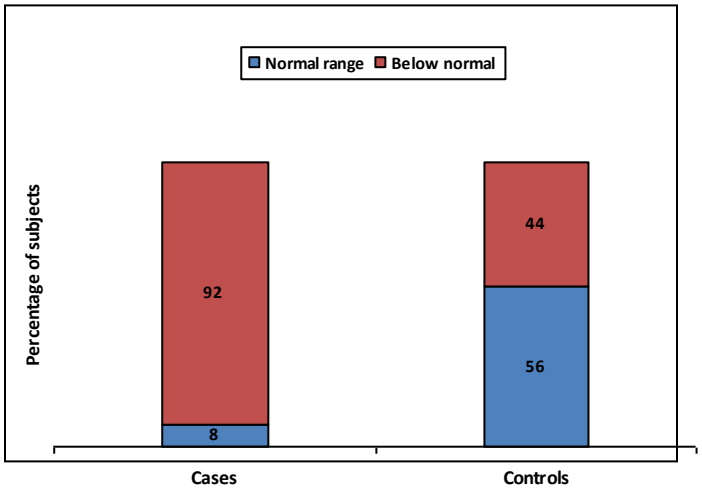


Figure 14: ADAMTS13 antigen levels in cases and controls

Median ADAMTS13 antigen level among cases was 0.14 µg/ml with a range of 0.02 to 0.77 µg/ml. Median ADAMTS13 antigen level among controls was 0.54 µg/ml with a range of 0.02 to 1.43 µg/ml The difference was statistically significant (p value-0.004) (Figure 15}

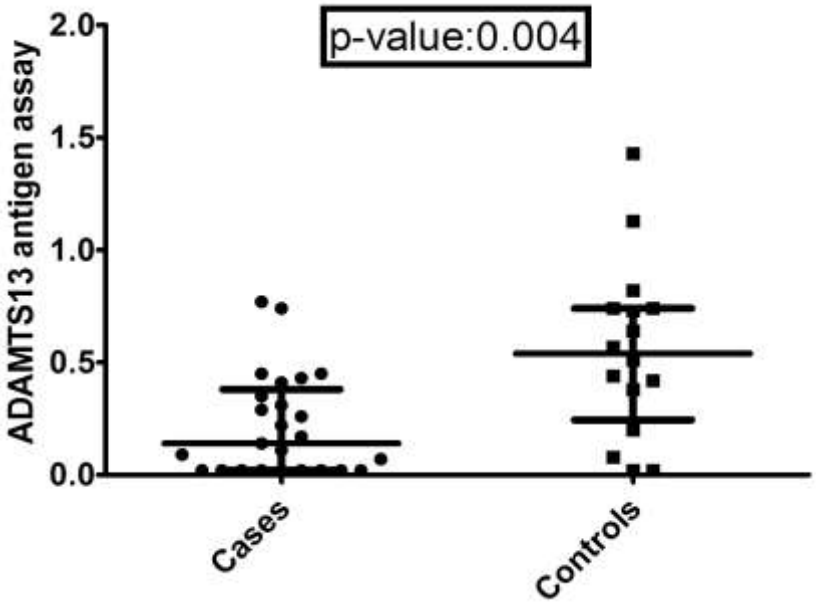


Figure 15: ADAMTS13 antigen levels in cases and controls

Significantly low ADAMTS13 antigen in Child's A NCIPH patients vs. Child's A controls

The ADAMTS13 antigen level among the NCIPH patients in Child's A status was compared with that of the controls in Child's A status. Median antigen level among cases (n=21) was 0.17 µg/ml, range 0.02 to 0.77 µg/ml. Median antigen level among controls (n=12) was 0.6 µg/ml, range 0.68 to 1.13 µg/ml. The difference was statistically significant (p value- 0.001)

No significant correlation between ADAMTS13 activity and MELD score in both cases and controls:

Correlation between ADAMTS13 activity and MELD score was calculated both in cases and controls using spearman correlation coefficient test. There was no significant correlation noted in both the groups (cases -spearman ρ=0.119, p value=0.617, controls - spearman ρ= -0.089, p value=0.773)

No significant correlation between ADAMTS13 activity and HVPG:

Correlation between ADAMTS13 activity and HVPG was calculated for cases with available HVPG, using spearman correlation coefficient test. There was no significant correlation noted (spearman ρ=0.125, p value-0.715)

DISCUSSION

The present study is a case control study to look for association between bacterial translocation and NCIPH. The study also aimed at assessing the ADAMTS13 activity in NCIPH patients. The study was initiated with a hypothesis that in NCIPH, underlying gut disorders and the resulting bacterial translocation trigger microangiopathy in the presence of low ADAMTS13 activity in the portal microcirculation.

We observed a high prevalence of celiac disease among NCIPH patients when compared to the general population. Population studies in India have shown prevalence of celiac disease in general population to be ranging from 0.3 to 1%(65,66), whereas in our study subjects, we found a 9.4% prevalence of celiac disease among the cases. This high prevalence of celiac disease is consistent with the findings of Eapen at al who documented 16% prevalence of celiac disease among NCIPH patients(4).

Anti tTG antibody positivity was about 30% in both NCIPH patients and in controls. Vecchi et al and Villalta et al documented a high prevalence of anti tTG positivity (upto 33%) among liver disease patients(67,68). They found the positivity to be generally false positive, partly related to hypergammaglobulinemia and associated with more advanced liver disease. This may explain the positive results of anti tTG antibody among our control group. In a previous study from our centre, 59 patients with hepatitis B or hepatitis C related chronic liver disease were tested for anti tTG antibody and 16 out of 59 were found to be positive (27%), a total of 28 underwent duodenal biopsy and none of them had villous atrophy. It is less likely for the positive serology to be false positive among NCIPH cases, since we were able to document villous atrophy in 3 out of 7 patients who were positive for celiac serology. On gluten free diet, anti tTG antibody

became negative in four patients, and in one another patient there was a significant reduction in titer.

One of the NCIPH patients negative for anti tTG antibody had mild villous atrophy in duodenal biopsy. This suggests there may be other gut disorders associated with NCIPH. Though there are many hypotheses for the pathogenesis of NCIPH, the exact etiology is unknown. The current study is in favor that gut disorders may be yet another association with NCIPH and to be looked for in this group.

Gut disorders are commonly associated with altered intestinal permeability(6,37,38) which in turn can lead to bacterial translocation(7). So we decided to look for evidence to support bacterial translocation in NCIPH patients. Generally demonstration of peripheral blood bacterial DNA by PCR method is considered to be the marker of bacterial translocation(44). Measurement of intestinal permeability by Lactulose/ Mannitol ratio testing is a sensitive and a specific marker of leaky gut and this can serve as an indirect measure for bacterial translocation(7). Presence of peripheral blood bacterial DNA is commonly associated with high levels of inflammatory markers like TNF alpha and interleukin 6(51), so we also looked for plasma TNF alpha levels in cases and controls.

Due to technical difficulties, peripheral blood bacterial DNA estimation was not accomplished in our study subjects. This was due to possible contamination leading to false positive results which was due to the high sensitive nature of the test method used. It is a widely accepted fact that, to maintain high sensitivity and specificity together in bacterial DNA estimation by PCR is challenging(69)

We observed a reasonably preserved intestinal permeability in both cases and controls. Nearly 20% of study subjects had high L/M ratio (≥0.086). Among controls, this is consistent with previous reports. Scarpellini et al demonstrated altered intestinal permeability among liver cirrhosis patients, in the range of about 20% in CTP A, 40% in CTP B and 75% in CTP C patients(45). In our study controls, most of them were in CTP A status (13 out of 16 patients) and so a 20% prevalence of abnormal intestinal permeability is acceptable. Among NCIPH patients, there is no available data on altered intestinal permeability. Celiac disease is generally accepted to have abnormal IP; Cobden et al demonstrated that L/M ratio testing has a sensitivity and accuracy as a screening test to detect celiac disease(6). In our study cases, only one of the three celiac disease patients underwent L/M ratio testing and was found to have abnormal test result. There were four more NCIPH cases with abnormal IP, 2 of them had chronic duodenitis in D2 biopsy. The reason for altered IP among NCIPH needs evaluation. This may be due to gut disease other than celiac disease.

We hypothesized an association between bacterial translocation and NCIPH, and we considered leaky status of the gut to be the cause for bacterial translocation. However we were not able to demonstrate a higher prevalence of abnormal IP among our study cases. Mechanisms of bacterial translocation include intestinal bacterial overgrowth, leaky gut and poor immune response(34,49,70) There may be additional contributors for bacterial translocation, however disappointingly we were not able to look for peripheral blood bacterial DNA to document bacterial translocation.

We also looked for TNF alpha levels, and we found it to be undetectable in both cases and controls. If it was high among the NCIPH patients, it would have supported the association between bacterial translocation and the disease.

Most important finding of the present study is the significantly low plasma ADAMTS13 activity and antigen levels among NCIPH patients when compared to hepatitis B/C related chronic liver disease patients. Uemura et al found a declining ADAMTS13 activity with worsening liver dysfunction among liver cirrhosis patients(11) However this does not explain the low ADAMTS13 activity among NCIPH patients without severe liver disease as per MELD score. Maximum MELD score among cases tested for ADAMTS13 activity was 13 and 75% of cases had low activity. When we excluded patients with Child's B status and analyzed the data for Child's A cases and controls, we found a significantly low ADAMTS13 activity and antigen among cases. Two patients with severely low activity of <2% were in CTP A status.

Above findings with regard to ADAMTS13 and NCIPH is consistent with that of Mackie et al(12). In the study by Mackie et al, 18 NCIPH patients were compared with matched controls. Low ADAMTS activity was noted in all the cases and the median value was significantly lower than the controls. The authors were successful in demonstrating the deficiency using multiple methods of ADAMTS assay. In this study there was a trend towards inverse correlation between ADAMTS levels and MELD score. In our study we found no correlation between ADAMTS13 levels and MELD score/ HVPG.

Severely low ADAMTS13 activity of <5% of normal is generally considered to be specific for TTP(60) However similar severe deficiency can happen in advanced end

stage liver cirrhosis as shown by Uemura et al. Herein we document severely low activity among NCIPH patients with well preserved liver function. This early occurrence of low ADAMTS13 activity suggests it to be a cause rather than an effect of the disease. The causes for low ADAMTS13 activity can be either decreased synthesis or rapid clearance or presence of inhibitors. Mackie et al looked for presence of antibodies/ inhibitors to ADAMTS13 and did not detect any significant factors. They also observed that after infusing FFP, there was a rise in ADAMTS13 activity with a good half life suggesting there is no rapid clearance happening. Authors proposed the cause for low ADAMTS13 activity to be due to synthetic dysfunction. Abnormally unresponsive stellate cells may be responsible for the low ADAMTS13 activity and the absence of hepatic fibrosis.

Constitutive low expression of ADAMTS13 would result in lowest ADAMTS13 levels in portal circulation making the portal vasculature at risk for microthrombi formation. In such a situation, when ultralrge vWF multimers are exposed at the surface of damaged endothelium, platelet aggregation and microangiopathy set in. Endothelial injury is more likely to be due to gut derived factors since the pathology is confined to portal circulation.

Abnormal vWF :ADAMTS ratio is documented to be important in the progression of liver injury in various conditions like acute on chronic liver failure, graft dysfunction following liver transplantation and in alcoholic liver disease(62,63) Whereas in NCIPH, the abnormal ratio may be the initiating factor in the pathogenesis.

CONCLUSIONS

- Most of the NCIPH patients had shrunken liver by imaging and almost half of them had a high HVPG suggesting that liver biopsy is essential to differentiate NCIPH from cryptogenic cirrhosis.
- Prevalence of celiac disease among NCIPH patients was noted to be 9.4% which is much higher than that reported among general population (upto 1%)
- Intestinal permeability was preserved in majority of patients both among cases and controls
- There was no rise in plasma TNF alpha levels both in cases and controls
- ADAMTS13 activity was found to be significantly low in NCIPH patients than in controls
- ADAMTS13 antigen level was found to be significantly low in NCIPH patients than in controls
- Among patients with compensated liver disease (CTP A status) there was a significantly low ADAMTS13 activity and antigen level among NCIPH patients in comparison with controls.
- There was no correlation between ADAMTS13 activity and MELD score or HVPG.

BIBLIOGRAPHY

1. Madhu K, Avinash B, Ramakrishna B, Eapen CE, Shyamkumar NK, Zachariah U, et al. Idiopathic non-cirrhotic intrahepatic portal hypertension: common cause of cryptogenic intrahepatic portal hypertension in a Southern Indian tertiary hospital. Indian J Gastroenterol. 2009 Jun;28(3):83–7.

2. Goel A, Ramakrishna B, Madhu K, Zachariah U, Ramachandran J, Keshava SN, et al. Idiopathic noncirrhotic intrahepatic portal hypertension is an ongoing problem in India. Hepatology. 2011;54(6):2275–6.

3. Saigal S, Nayak NC, Jain D, Kumaran V, Mohanka R, Saraf N, et al. Non-cirrhotic portal fibrosis related end stage liver disease in adults: evaluation from a study on living donor liver transplant recipients. Hepatology International. 2011 Jan 12;5(4):882–9.

4. Eapen CE, Nightingale P, Hubscher SG, Lane PJ, Plant T, Velissaris D, et al. Non-cirrhotic Intrahepatic Portal Hypertension: Associated Gut Diseases and Prognostic Factors. Digestive Diseases and Sciences. 2010 May 25;56(1):227–35.

5. Hillaire S, Bonte E, Denninger M-H, Casadevall N, Cadranel J-F, Lebrec D, et al. Idiopathic non-cirrhotic intrahepatic portal hypertension in the West: a re-evaluation in 28 patients. Gut. 2002 Aug 1;51(2):275–80.

6. Cobden I, Rothwell J, Axon AT. Intestinal permeability and screening tests for coeliac disease.Gut. 1980 Jun 1;21(6):512–8.

7. Balzan S, De Almeida Quadros C, De Cleva R, Zilberstein B, Cecconello I. Bacterial translocation: Overview of mechanisms and clinical impact. Journal of Gastroenterology and Hepatology. 2007;22(4):464–71.

8. Bellot P, García-Pagán JC, Francés R, Abraldes JG, Navasa M, Pérez-Mateo M, et al. Bacterial DNA translocation is associated with systemic circulatory abnormalities and intrahepatic endothelial dysfunction in patients with cirrhosis. Hepatology. 2010 Dec;52(6):2044–52.

9. Aoyama T, Paik Y-H, Seki E. Toll-Like Receptor Signaling and Liver Fibrosis. Gastroenterol Res Pract;2010. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2913673/

10. Uemura M, Tatsumi K, Matsumoto M, Fujimoto M, Matsuyama T, Ishikawa M, et al. Localization of ADAMTS13 to the stellate cells of human liver. Blood. 2005 Aug 1;106(3):922–4.

11. Uemura M, Fujimura Y, Matsumoto M, Ishizashi H, Kato S, Matsuyama T, et al. Comprehensive analysis of ADAMTS13 in patients with liver cirrhosis. Thrombosis and Haemostasis . 2008 May 9 ; Available from: http://www.schattauer.de/index.php?id=1214&doi=10.1160/TH08-01-0006

12. Mackie I, Eapen CE, Neil D, Lawrie AS, Chitolie A, Shaw JC, et al. Idiopathic Noncirrhotic Intrahepatic Portal Hypertension is Associated with Sustained ADAMTS13 Deficiency. Digestive Diseases and Sciences. 2011 May 15;56(8):2456–65.

13. Ibarrola C, Colina F. Clinicopathological features of nine cases of non-cirrhotic portal hypertension: current definitions and criteria are inadequate. Histopathology. 2003 Mar;42(3):251–64.

14. Sarin SK, Kumar A, Chawla YK, Baijal SS, Dhiman RK, Jafri W, et al. Noncirrhotic portal fibrosis/idiopathic portal hypertension: APASL recommendations for diagnosis and treatment. Hepatol Int. 2007 Sep;1(3):398–413.

15. RAMALINGASWAMI V, WIG KL, SAMA SK. Cirrhosis of the liver in northern India. A clinicopathologic study. Arch. Intern. Med. 1962 Sep;110:350–8.

16. Imanaga H, Yamamoto S, Kuroyanagi Y. Surgical Treatment of Portal Hypertension According to State of Intrahepatic Circulation. Ann. Surg. 1962 Jan;155(1):42–50.

17. Mikkelsen WP, Edmondson HA, Peters RL, Redeker AG, Reynolds TB. Extra- and intrahepatic portal hypertension without cirrhosis (hepatoportal sclerosis). Ann. Surg. 1965 Oct;162(4):602–20.

18. Pandechandana, Sarin SK. Non-Cirrhotic Portal Fibrosis: A Clinical Profile of 366 Patients. American Journal of Gastroenterology . 2006 ;101(S 191). Available from: http://www.nature.com/ajg/journal/v101/n2s/full/ajg2006561a.html

19. Kingham JGC, Levison DA, Stansfeld AG, Dawson AM. Non-cirrhotic Intrahepatic Portal Hypertension: A Long Term Follow-up Study. QJM. 1981 Jun 20;50(3):259–68.

20. Dhiman RK, Chawla Y, Vasishta RK, Kakkar N, Dilawari JB, Trehan MS, et al. Non-cirrhotic portal fibrosis (idiopathic portal hypertension): experience with 151 patients and a review of the literature. J. Gastroenterol. Hepatol. 2002 Jan;17(1):6–16.

21. Nayak NC, Ramalingaswami V. Obliterative portal venopathy of the liver. Associated with so-called idiopathic portal hypertension or tropical splenomegaly.Arch Pathol. 1969 Apr;87(4):359–69.

22. Okudaira M, Ohbu M, Okuda K. Idiopathic portal hypertension and its pathology. Semin. Liver Dis. 2002 Feb;22(1):59–72.

23. Nakanuma Y, Tsuneyama K, Ohbu M, Katayanagi K. Pathology and pathogenesis of idiopathic portal hypertension with an emphasis on the liver. Pathol. Res. Pract. 2001;197(2):65–76.

24. Ludwig J, Hashimoto E, Obata H, Baldus WP. Idiopathic portal hypertension; a histopathological study of 26 Japanese cases.Histopathology. 1993 Mar;22(3):227–34.
25. Sarin SK, Kumar A. Noncirrhotic Portal Hypertension. Clinics in Liver Disease.2006 Aug;10(3):627–51.

26. Kono K, Ohnishi K, Omata M, Saito M, Nakayama T, Hatano H, et al. Experimental portal fibrosis produced by intraportal injection of killed nonpathogenic Escherichia coli in rabbits. Gastroenterology. 1988 Mar;94(3):787–96.

27. Saito K, Nakanuma Y, Takegoshi K, Ohta G, Obata Y, Okuda K, et al. Non-specific immunological abnormalities and association of autoimmune diseases in idiopathic portal hypertension. A study by questionnaire.Hepatogastroenterology. 1993 Apr;40(2):163–6.

28. Datta DV, Mitra SK, Chhuttani PN, Chakravarti RN.Chronic oral arsenic intoxication as a possible aetiological factor in idiopathic portal hypertension (non-cirrhotic portal fibrosis) in India.Gut. 1979 May;20(5):378–84.

29. Smith PM, Crossley IR, Williams DM. Portal hypertension in vinyl-chloride production workers. Lancet. 1976 Sep 18;2(7986):602–4.

30. Sharma BC, Bhasin DK, Nada R. Association of celiac disease with non-cirrhotic portal fibrosis. Journal of Gastroenterology and Hepatology. 2006;21(1):332–4.

31. Cancado ELR, Medeiros DM, Deguti MM, Dos Santos MSC, De Mello ES, Vendramini MBG, et al. Celiac disease associated with nodular regenerative hyperplasia, pulmonary abnormalities, and IgA anticardiolipin antibodies. J. Clin. Gastroenterol. 2006 Feb;40(2):135–9.

32. Austin A, Campbell E, Lane P, Elias E. Nodular regenerative hyperplasia of the liver and coeliac disease: potential role of IgA anticardiolipin antibody. Gut. 2004 Jul;53(7):1032–4.

33. Zamani F, Amiri A, Shakeri R, Zare A, Mohamadnejad M. Celiac disease as a potential cause of idiopathic portal hypertension: a case report. Journal of Medical Case Reports. 2009 Feb 16;3(1):68.

34. Berg RD. Mechanisms confining indigenous bacteria to the gastrointestinal tract. Am J ClinNutr. 1980 Nov 1;33(11):2472–84.

35. Madara JL. Warner-Lambert/Parke-Davis Award lecture.Pathobiology of the intestinal epithelial barrier. Am. J. Pathol. 1990 Dec;137(6):1273–81.

36. Arrieta MC, Bistritz L, Meddings JB. Alterations in intestinal permeability.Gut. 2006 Oct;55(10):1512–20.

37. Kumar S, Ghoshal UC, Jayalakshmi K, Roy R, Misra A, Khetrapal CL. Abnormal small intestinal permeability in patients with idiopathic malabsorption in tropics (tropical sprue) does not change even after successful treatment. Dig. Dis. Sci. 2011 Jan;56(1):161–9.
38. Wyatt J, Oberhuber G, Pongratz S, Püspök A, Moser G, Novacek G, et al. Increased gastric and intestinal permeability in patients with Crohn's disease. Am. J. Gastroenterol. 1997 Oct;92(10):1891–6.

39. Gutiérrez A, Francés R, Amorós A, Zapater P, Garmendia M, Ndongo M, et al. Cytokine association with bacterial DNA in serum of patients with inflammatory bowel disease.Inflamm. Bowel Dis. 2009 Apr;15(4):508–14.

40. Silva MA, Jury J, Sanz Y, Wiepjes M, Huang X, Murray JA, et al. Increased bacterial translocation in gluten-sensitive mice is independent of small intestinal paracellular permeability defect. Dig. Dis. Sci. 2012 Jan;57(1):38–47.

41. Ono S, Tsujimoto H, Yamauchi A, Hiraki S, Takayama E, Mochizuki H. Detection of microbial DNA in the blood of surgical patients for diagnosing bacterial translocation. World J Surg. 2005 Apr;29(4):535–9.

42. Kane TD, Johnson SR, Alexander JW, Babcock GF, Ogle CK. Detection of intestinal bacterial translocation using PCR. J. Surg. Res. 1996 Jun;63(1):59–63.

43. Wen L, Tang W, Jiang Z, Wang S, Liang C, Zhang Y. [Detection of bacterial DNA in blood with three pairs of primers]. Zhongguo Yi XueKeXue Yuan XueBao. 2000 Dec;22(6):592–4.

44. Such J. Detection and identification of bacterial DNA in patients with cirrhosis and culture-negative, nonneutrocytic ascites. Hepatology. 2002 Jul;36(1):135–41.

45. Scarpellini E, Valenza V, Gabrielli M, Lauritano EC, Perotti G, Merra G, et al. Intestinal Permeability in Cirrhotic Patients With and Without Spontaneous Bacterial Peritonitis: Is the Ring Closed? The American Journal of Gastroenterology. 2009 Oct 20;105(2):323–7.

46. Grootjans J. Non-invasive assessment of barrier integrity and function of the human gut. World Journal of Gastrointestinal Surgery. 2010;2(3):61.

47. Dastych M, Dastych M, Novotná H, Číhalová J. Lactulose/Mannitol Test and Specificity, Sensitivity, and Area under Curve of Intestinal Permeability Parameters in Patients with Liver Cirrhosis and Crohn's Disease. Digestive Diseases and Sciences. 2008 Mar 5;53(10):2789–92.

48. D'Ettorre G, Douek D, Paiardini M, Ceccarelli G, Vullo V. Microbial Translocation and Infectious Diseases: What Is the Link? Int J Microbiol;2012. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3471423/

49. Wiest R, Garcia-Tsao G. Bacterial translocation (BT) in cirrhosis. Hepatology. 2005 Mar;41(3):422–33.

50. Guarner C, González-Navajas JM, Sánchez E, Soriando G, Francés R, Chiva M, et al. The detection of bacterial DNA in blood of rats with CCl4-induced cirrhosis with ascites represents episodes of bacterial translocation. Hepatology. 2006 Sep;44(3):633–9.

51. González-Navajas JM, Bellot P, Francés R, Zapater P, Muñoz C, García-Pagán JC, et al. Presence of bacterial-DNA in cirrhosis identifies a subgroup of patients with marked inflammatory response not related to endotoxin. Journal of Hepatology. 2008 Jan;48(1):61–7.

52. Zapater P, Francés R, González-Navajas JM, De la Hoz MA, Moreu R, Pascual S, et al. Serum and ascitic fluid bacterial DNA: A new independent prognostic factor in noninfected patients with cirrhosis. Hepatology. 2008 Dec;48(6):1924–31.

53. El-Naggar MM, Khalil E-SA-M, El-Daker MAM, Salama MF. Bacterial DNA and its consequences in patients with cirrhosis and culture-negative, non-neutrocytic ascites.Journal of Medical Microbiology. 2008 Dec 1;57(12):1533–8.

54. Seki E, Schnabl B. Role of innate immunity and the microbiota in liver fibrosis: crosstalk between the liver and gut. J. Physiol. (Lond.). 2012 Feb 1;590(Pt 3):447–58.

55. Hartmann P, Chen W-C, Schnabl B. The intestinal microbiome and the leaky gut as therapeutic targets in alcoholic liver disease. Front Physiol. 2012;3:402.

56. Ilan Y. Leaky gut and the liver: A role for bacterial translocation in nonalcoholic steatohepatitis. World Journal of Gastroenterology. 2012;18(21):2609.

57. Lämmle B, Kremer Hovinga JA, Alberio L. Thrombotic thrombocytopenic purpura. Journal of Thrombosis and Haemostasis. 2005;3(8):1663–75.

58. Moore JC, Hayward CPM, Warkentin TE, Kelton JG. Decreased von Willebrand factor protease activity associated with thrombocytopenic disorders. Blood. 2001 Sep 15;98(6):1842–6.

59. Mannucci PM, Canciani MT, Forza I, Lussana F, Lattuada A, Rossi E. Changes in health and disease of the metalloprotease that cleaves von Willebrand factor. Blood. 2001 Nov 1;98(9):2730–5.

60. Bianchi V, Robles R, Alberio L, Furlan M, Lämmle B. Von Willebrand factor-cleaving protease (ADAMTS13) in thrombocytopenic disorders: a severely deficient activity is specific for thrombotic thrombocytopenic purpura. Blood. 2002 Jul 15;100(2):710–3.

61. Uemura M, Fujimura Y, Ko S, Matsumoto M, Nakajima Y, Fukui H. Pivotal role of ADAMTS13 function in liver diseases. International Journal of Hematology. 2010 Jan 7;91(1):20–9.

62. Matsuyama T, Uemura M, Ishikawa M, Matsumoto M, Ishizashi H, Kato S, et al. Increased von Willebrand factor over decreased ADAMTS13 activity may contribute to the development of liver disturbance and multiorgan failure in patients with alcoholic hepatitis. Alcohol. Clin. Exp. Res. 2007 Jan;31(1 Suppl):S27–35.

63. Ko S, Okano E, Kanehiro H, Matsumoto M, Ishizashi H, Uemura M, et al. Plasma ADAMTS13 activity may predict early adverse events in living donor liver transplantation: observations in 3 cases. Liver Transpl. 2006 May;12(5):859–69.

64. Ferlitsch M, Reiberger T, Hoke M, Salzl P, Schwengerer B, Ulbrich G, et al. Von Willebrand factor as new noninvasive predictor of portal hypertension, decompensation and mortality in patients with liver cirrhosis. Hepatology. 2012 Oct;56(4):1439–47.

65. Sood A, Midha V, Sood N, Avasthi G, Sehgal A. Prevalence of celiac disease among school children in Punjab, North India. J. Gastroenterol. Hepatol. 2006 Oct;21(10):1622–5.

66. Makharia GK, Verma AK, Amarchand R, Bhatnagar S, Das P, Goswami A, et al. Prevalence of celiac disease in the northern part of India: a community based study. J. Gastroenterol. Hepatol. 2011 May;26(5):894–900.

67. Vecchi M, Folli C, Donato MF, Formenti S, Arosio E, De Franchis R. High rate of positive anti-tissue transglutaminase antibodies in chronic liver disease. Role of liver decompensation and of the antigen source. Scand. J. Gastroenterol. 2003 Jan;38(1):50–4.

68. Villalta D, Crovatto M, Stella S, Tonutti E, Tozzoli R, Bizzaro N. False positive reactions for IgA and IgG anti-tissue transglutaminase antibodies in liver cirrhosis are common and method-dependent. Clin.Chim.Acta. 2005 Jun;356(1-2):102–9.

69. Philipp S, Huemer HP, Irschick EU, Gassner C. Obstacles of Multiplex Real-Time PCR for Bacterial 16S rDNA: Primer Specifity and DNA Decontamination of Taq Polymerase. Transfus Med Hemother. 2010 Feb;37(1):21–8.

70. Pinzone MR, Celesia BM, Di Rosa M, Cacopardo B, Nunnari G. Microbial translocation in chronic liver diseases.Int J Microbiol. 2012;2012:694629.

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APPENDIX

Performa

Name:

Age:

Sex:

Contact Address:

Email:

Phone No.:

Hospital No.:

Height:

Weight:

BMI:

Kuppusamy's Socio economic score:

Case/Control:

Diagnosis:

Ascites:

CTP score:

MELD score:

UGI scopy(Variceal status):

USG abdomen:

Bacterial DNA:

Peripheral blood:

Ascitic Fluid:

L/M ratio:

ADAMTS13 level:

VWF level:

Liver Biopsy:

Liver Biopsy report:

For Cases:

1. Viral Markers:
HBSAg:
HCV antibody:
Total Core:

2. Significant Alcohol history:

3. Anti TTG antibody:

4. ANA:

5. Immunoglobulins:

6. Ferritin:

7. Ceruloplasmin:

8. Sugars:
AC:
PC:

9. Lipid profile:
Total Cholesterol:
Triglycerides:
HDL:
LDL:

10. Doppler of hepatic veins/ IVC:

11. Vitamin B12 level:

Master sheet

Cases:

NO	NAME	hosp. no	AGE	SEX	state	income	education
1	UthiraKumar	773446D	23	M	T.N	5000	STUDENT
2	Indria Deo narayan Tiwary	153886D	62	M	JHARKAND	5000	10TH
3	Cheran	898674D	26	M	KARNATAKA	1,00,000	B.E
4	Sudansu Sarkar	688818D	46	M	W.B	3000	8TH
5	Arbinda Kumar	130559D	36	M	BIHAR	15,000	10TH
6	Anitha Mary	006861D	36	F	T.N	4500	9TH
7	Kalaiselvi.R	531858C	39	F	T.N	6000	8TH
8	Kamaraj.G	496796B	47	M	T.N	3,000	10TH
9	Rajini.M	766324D	24	F	T.N	3000	5TH
10	Balbir Kaur	750100D	26	F	JHARKAND	8000	5TH
11	Dinesh Chandrasarkar	830578C	27	M	W.B	10,000	GRADUAT
12	Akshai	000139C	29	M	T.N	1,00,000	B.E
13	Satheesh kumar	713415D	31	M	T.N	30,000	10TH
14	Bandhu Dhari Singh	251324D	40	M	BIHAR	1,00,000	B.E
15	Rajendran	663103A	57	M	T.N	12,000	POSTGRA
16	Megha Ramachandran	766047D	29	F	T.N	20,000	CLERK
17	VirendraPrasad	741937D	50	M	CHATTISGA	20,000	BUSINESS
18	VIJAYAKUMAR	448809B	33	M	BIHAR	3000	DIPLOMA
19	SHYAMAL DUTTA	018241F	40	M	BANGLADES	3000	8TH
20	PRASANTH	757536D	21	M	T.N	3000	GRADUAT
21	VINODKUMAR	496133D	33	M	T.N	100,000	DIPLOMA
22	MALLIGA	065061D	49	F	T.N	1000	ILLITERAT
23	ALOM BADSHA	011442F	36	M	BANGLADES	10,000	10TH
24	SANTHOSH KUMAR ROUT	272679C	37	M	W.B	10,000	9TH
25	RANI	672156B	58	F	T.N	1,000	ILLITERAT
26	YAJUM GARAM	255012D	39	F	ARUNACHAI	50,000	12TH
27	ASHOK BANERJEE	157026F	54	M	W.B	50,000	POSTGRA
28	SADHAN GORAI	875269C	28	M	W.B	3000	10TH
29	RAJAMMA	004578D	49	F	A.P	3000	ILLITERAT
30	ANJANI PRASAD	193760F	25	M	A.P	6000	10TH
31	NITU KUMARI	503815D	23	F	JHARKAND	20,000	11TH
32	VEENA PRATHIBA P.	652528D	30	F	T.N	4,00,000	POSTGRA
33	KUPPILI ESWARAMMA	223043F	27	F	A.P	15,000	5TH

job	KUPPUSA	ht	wt	BMI	ASCITES	CTP	MELD	VARICES
NIL	III	166	58	21	MILD	B-7	10	SEC EVL, SMALL VARICES
POST MASTER	III	155	57	23.7	NO	A-5	10	SEC EVL,GRADE 1X3,SMA
ENGINEER	I	178	90	28.4	NO	A-5	10	GRADEII-III VARICES,NO B
FARMER	III	155	47	19.6	NO	B-7	12	SEC EVL, GRADEII-III
CONSTABLE	II	178	92	29	NO	A-5	10	SMALL, NON BLEEDER
NIL	IV	153	53	22.6	NO	A-5	6	SMALL, NON BLEEDER
NIL	IV	150	41.5	18.4	MILD	A-5	10	NO VARICES. NO BLEED
FARMER	III	160	50	19.5	MILD	A-5	8	SMALL, NON BLEEDER
LABOURER	IV	148	42	19.2	NO	A-5	10	VARICES,NO BLEED
NIL	IV	149	50	22.5	NO	A-5	8	SEC EVL
PANCHAYAT	II	163	52	19.6	NO	A-5	9	SEC EVL
ENGINEER	I	170	79	27.3	NO	B-7	11	POST MESOCAVALSHUNT
PHOTOGRAPHE	II	160	64	25	NO	A-5	11	SMALL, NON BLEEDER
ENGINEER	I	180	107	33	NO	B-7	15	NO VARICES
HEADMASTER	II	164	69	25.7	NO	A-5	7	SECEST
GRADUATE	II	148	50	22.8	NO	A-5	7	SMALL, NON BLEEDER
12TH	II	161	55	19.7	MILD	B-7	13	SEC EVL
TEACHER	III	170	65	22.5	NO	A-5	10	SEC EVL
SHOP OWNER	III	170	58	20.1	NO	A-5	8	PRIMARY EVL
STUDENT	III	165	51.8	19	NO	A-6	11	SMALL, NON BLEEDER
BUSINESS	II	175	88	28.7	NO	A-5	9	LARGE,NON BLEEDER
LABOURER	IV	149	36.6	16.5	NO	A-5	8	SEC EST
CASHIER	II	162	59	22.5	NO	A-5	8	SEC EVL
SECURITY	II	172	86.3	29.2	NO	A-5	7	SEC EVL
LABOURER	IV	155	88	36.6	NO	A-5	7	SEC EST
CLERK	II	152	61	26.4	NO	A-5	11	SEC EVL
PRINCIPAL	II	168	53	18.8	NO	A-6	13	PRIMARY EVL
SHOP OWNER	III	167	60	21.5	NO	A-6	11	SEC EVL
NIL	IV	155	53	22.1	NO	A-5	9	SMALL, NON BLEEDER
SECURITY	III	157	55	22.3	NO	A-5	9	SEC EVL
NIL	II	157	46	18.7	MILD	A-6	8	SEC EVL
TEACHER	II	153	53	22.6	NO	A-5	7	NO VARICES
NIL	III	157	44	17.85	NO	A-5	12	NO VARICES

PLATELET	USG LIVER	IGG	igA	HVPG	L	M	L/M
103,000	SHRUNKEN LIV	2421/398/281	398		5	0.776	18.381	0.0422
62,000	SHRUNKEN LIV	2782/716/108	716		11	0.459	2.675	0.1716
73,000	SHRUNKEN LIV	1677/263/59	263	ND		0.586	12.419	0.0472
60,000	SHRUNKEN LIV	1697/256/110	256	ND		0.435	11.354	0.0383
60,000	SHRUNKEN LIV	2434/409/49	409		10	0.673	1.866	0.3607
344,000	NORMAL SIZE	ND		ND		0.491	19.467	0.0252
38,000	NORMAL SIZE	ND		ND		0.381	14.506	0.0263
60,000	NORMAL SIZE	ND		ND		0.327	17.412	0.0189
35,000	SHRUNKEN LIV	ND		ND		0.175	6.007	0.0291
55,000	NORMAL SIZE	ND			3	0.658	7.356	0.0895
45,000	NORMAL SIZE	1798/258/94	258	ND		0.236	2.594	0.091
92,000	SHRUNKEN LIV	1437/426/110	426	ND		invalid	invalid	invalid
60,000	NORMAL SIZE	2164/251/115	251		3			ND
120,000	SHRUNKEN LIV	ND		ND				ND
233,000	NORMAL SIZE	ND		ND				ND
104,000	SHRUNKEN LIV	1434/334/168	334		10	invalid	invalid	invalid
52,000	SHRUNKEN LIV	ND		ND				ND
134,000	SHRUNKEN	ND		ND		0.145	6.976	0.0208
104,000	SHRUNKEN	2833/454/116	454		2	0.245	8.614	0.0284
46,000	SHRUNKEN				2	0.247	14.257	0.0173
115,000	SHRUNKEN	1599/312/199	312		6	0.088	8.574	0.0102
47,000	NORMAL	1957/388/156	388		4	0.271	14.365	0.0189
99000	SHRUNKEN	ND			7	0.218	6.49	0.0336
120,000	SHRUNKEN	ND		ND				ND
255,000	SHRUNKEN	ND		ND		0.534	22.084	0.0242
70,000	SHRUNKEN	2415/259/88	259		2			0.1231
30,000	SHRUNKEN	2053/588/73	588	ND				ND
29,000	NORMAL	ND		ND				0.0313
37,000	SHRUNKEN LIV	1762/283/126	283		9			0.056
62,000	SHRUNKEN	ND			15			0.00574
63,000	SHRUNKEN LIV	1789/325/32	325		10			invalid
63,000	NORMAL	ND		ND				0.0293
35,000	NORMAL	1474/157/178	157	ND				ND

ADMTS 13 activity	ADAMTS 13 antige	TTG	D2 BIOPSY	ADAMTS A	ADAMTS 13 Ag
ND	0.02	NEGATIVE	CHRONIC DUODENITIS	18	30
>104.6	0.35	NEGATIVE	CHRONIC DUODENITIS	50	34
ND	ND	POSITIVE	NORMAL	22	63
>104.6	0.74	NEGATIVE	ND	65	46
16.84	0.11	POSITIVE	MODERATE VILLOUS AT	14	45
ND	0.22	NEGATIVE	ND	69	111
ND	0.17	ND	ND	13	63
12.74	0.02	POSITIVE	CHRONIC DUODENITIS	5	47
ND	ND	NEGATIVE	ND	5	67
52.87	0.29	POSITIVE	CHRONIC DUODENITIS	11	77
85.31	0.31	NEGATIVE	NORMAL	ND	
54.97	0.02	NEGATIVE	CHRONIC DUODENITIS	35	74
ND	0.02	POSITIVE	MODERATE VILLOUS AT	68	49
ND	0.02	NEGATIVE	ND	69	86
ND	ND	NEGATIVE	ND	44	104
12.54	0.02	POSITIVE	CHRONIC DUODENITIS		
ND	ND	POSITIVE	MILD VILLOUS ATROPHY		
41.51	0.02	NEGATIVE	NORMAL		
45.75	0.26	NEGATIVE	ND		
41.72	0.45	NEGATIVE	BRUNNER GLAND HYPERPLASIA		
44.10	0.77	NEGATIVE	ND		
2.00	0.41	NEGATIVE	ND		
ND	ND	NEGATIVE	CHRONIC DUODENITIS		
44.69	0.43	POSITIVE	ND		
>104.6	0.45	NEGATIVE	ND		
2.00	0.09	NEGATIVE	ND		
57.03	0.14	POSITIVE	ND		
14.63	0.02	NEGATIVE	MILDVILLOUSATROPHY		
>104.6	0.07	NEGATIVE	ND		
29.38	0.02	NEGATIVE	ND		
ND	ND	NEGATIVE	ND		
ND	ND	NEGATIVE	ND		
ND	ND	NEGATIVE	ND		

Controls:

NO	NAME	hosp. no	AGE	SEX	state	DIAGNOSI	income	education
1	Krishna Devi	916775D	47	F	BIHAR	HCV CLD	50,000	10TH
2	Satya Ranjan Das	841513D	48	M	W.B	HBV CLD	3000	7TH
3	Sideswar Prasad	485744D	43	M	JHARKHN	HBV CLD	3000	10TH
4	Amrito lal Das	903248D	48	M	W.B	HBV CLD	10,000	GRADUAT
5	Samburam Jana	407218D	36	M	W.B	HBV CLD	3000	8TH
6	Tarak Halder	877663D	46	M	JHARKHN	HBV CLD	3000	4TH
7	Bibhuta Sarkar	973972D	41	M	W.B	HBV CLD	8,000	8TH
8	Chandrasekhar Das	973748D	45	M	JHARKHN	HBV CLD	10,000	10TH
9	Pabitra maity	725751C	38	F	W.B	HBV CLD	15,000	12TH
10	Rajkumar Das	993579D	30	M	W.B	HBV CLD	4000	10TH
11	Ashish Kumar chowd	250599D	52	M	BANGLAD	HCV CLD	3,000	10TH
12	Wahid	932063D	37	M	JHARKHN	HBV CLD	4000	9TH
13	Dulal	642681D	24	M	BANGLAD	HBV CLD	3000	4TH
14	Rafikul	055069D	34	M	W.B	HBV CLD	3000	7TH
15	Sankar Rana	973728d	42	M	W.B	HBV CLD	3000	8TH
16	Mohan mondal	965035d	46	M	W.B	HCV CLD	5000	8TH
17	chelo tatup	986801d	63	M	ARUNACH	HBV CLD	10,000	NIL
18	biri takar	863531d	22	M	ARUNACH	HBV CLD	10,000	ENGINEEF
19	AFTAB UDDIN MOLI	866126D	35	M	W.B	HBV CLD	10,000	12TH
20	NAWAL KISHORE L	128888F	57	M	BIHAR	HBV CLD	5000	GRADUAT
21	HARINADHA REDD\	556880C	53	M	A.P	HBV CLD	4000	GRADUAT
22	MOHAMMED IQBAL	141085F	40	M	BANGLAD	HBV CLD	15,000	GRADUAT
23	PRASENJITH	956411D	17	M	W.B	HBV CLD	8,000	12TH
24	KASINATHAN	708572B	58	M	A.P	HBV CLD	1,000	10TH
25	KOKHAN DAS	876184D	31	M	W.B	HBV CLD	5,000	7TH

job	KUPPUSA	ht	wt	BMI	ASCITES	CTP	MELD	VARICES
NIL	II	140	43	21.9	NO	A-6	9	SMALL, NON BLE
LABOURE	IV	160	60	23.4	NO	A-6	11	SMALL, NON BLE
SHOP OW	III	155	56	23.3	NO	A-5	9	SEC EVL
CLERK	II	164	59	21.9	NO	A-5	10	SEC EVL
LABOURE	IV	165	60	22	NO	A-5	11	SEC EVL
LABOURE	IV	165	62		NO	A-6	9	SMALL, NON BLE
CLERK	III	155	54	22.8	MILD	B-8	11	SEC EVL
SHOP OW	II	155	56	23.3	NO	A-5	9	SEC EVL
RAILWAY	III	160	59	23	NO	A-5	8	SMALL, NON BLE
BARBER	IV	164	64	23.8	MILD	B-9	16	SMALL, NON BLE
FARMER	III	159	56	22.1	NO	A-5	10	SMALL, NON BLE
PAINTER	IV	171	69	23.6	MILD	B-9	15	SMALL, NON BLE
LABOURE	IV	165	71	26	NO	A-5	10	SMALL, NON BLE
FARMER	IV	172	62	20.9	NO	A-5	10	SMALL, NON BLE
SHOP OW	III	170	58	20.1	MILD- MO	B-7	13	SEC EVL
DRIVER	IV	165	58	21.3	NO	A-6	11	SMALL, NON BLE
FARMER	III	167	57	20.4	NO	A-5	9	SMALL, NON BLE
STUDENT	II	170	63	21.8	NO	A-6	8	SEC EVL
WORKSH	II	175	81	26.4	NO	A-5	11	SMALL, NON BLE
TEACHER	II	154	56	23.6	NO	A-5	11	SEC EVL
FARMER	III	170	58	20.1	NO	A-5	9	SMALL, NON BLE
BUSINESS	II	168	60	21.3	NO	B-7	10	SMALL, NON BLE
BUSINESS	II	150	43	19.1	NO	A-5	10	SMALL, NON BLE
FARMER	III	158	55	22	NO	A-5	9	SMALL, NON BLE
SHOP	III	175	82	26.7	NO	A-5	11	SMALL, NON BLE

PLATELET	USG LIVER	IGG	iga	L	M	L/M	ADMTS 13
120,000	SHRUNKEN LIVER	2754/548/314	548	0.139	28.392	0.0048	ND
24,000	SHRUNKEN LIVER	2658/534/76	534	1.185	5.559	0.2132	>104.6
90,000	SHRUNKEN LIVER	1779/367/65	367	0.231	5.507	0.0419	ND
60,000	NORMAL	2284/415/102	415	0.47	7.671	0.0613	ND
53,000	NORMAL	ND		0.391	4.995	0.0783	>104.6
142,000	NORMAL	2768/250/208	250	0.249	2.691	0.0925	ND
36,000	SHRUNKEN LIVER	ND		0.934	14.479	0.0645	ND
101,000	NORMAL	ND		1.132	50.953	0.0222	ND
170,000	NORMAL	1963/283/56	283	0.418	6.183	0.0676	ND
30,000	SHRUNKEN LIVER	2454/548/141	548	0.884	14.147	0.0625	15.77
81,000	SHRUNKEN LIVER	2307/479/124	479			ND	ND
39,000	SHRUNKEN LIVER	ND				ND	ND
69,000	NORMAL	ND				ND	ND
61,000	SHRUNKEN LIVER	ND				ND	>104.6
60,000	SHRUNKEN LIVER	ND				ND	ND
75,000	SHRUNKEN LIVER	ND				ND	>104.6
55,000	SHRUNKEN LIVER	ND				ND	<2
146,000	SHRUNKEN LIVER	1185/119/59	119			ND	>104.6
116,000	NORMAL	ND		0.585	9.151	0.064	96.20
30,000	SHRUNKEN LIVER	2223/304/168	304	0.288	3.69	0.0781	>104.6
75,000	SHRUNKEN LIVER					ND	ND
128,000	SHRUNKEN LIVER	2075/808/364	808	0.161	2.499	0.0645	57.84
26,000	SHRUNKEN LIVER	ND		0.202	6.369	0.0318	100.07
160,000	SHRUNKEN LIVER	ND		0.126	3.61	0.0349	>104.6
72,000	NORMAL	ND		0.702	5.448	0.1289	15.36

ADAMTS 1	TTG	D2 BIOPSY
0.74	POSITIVE	ND
0.64	NEGATIVE	ND
ND	NEGATIVE	ND
ND	NEGATIVE	ND
0.73	ND	ND
ND	NEGATIVE	ND
ND	ND	ND
ND	ND	ND
ND	ND	ND
0.44	ND	ND
ND	ND	ND
0.02	POSITIVE	ND
ND	NEGATIVE	ND
0.51	ND	ND
0.02	ND	MILD VILLOUS ATROPHY, CHRONIC DUODENITIS
1.13	ND	ND
0.74	ND	ND
0.57	ND	ND
0.08	ND	ND
0.20	ND	ND
ND	ND	ND
1.43	NEGATIVE	ND
0.42	NEGATIVE	ND
0.38	ND	ND
0.82	POSITIVE	ND